F. ENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 21 February 2001 (21.02.01)	
International application No. PCT/IL00/00332	**Applicant's or agent's file reference** 9956 PCT
International filing date (day/month/year) 07 June 2000 (07.06.00)	**Priority date** (day/month/year) 07 June 1999 (07.06.99)
Applicant WARSHAWSKY, Abraham et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   04 January 2001 (04.01.01)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Claudio Borton

Telephone No.: (41-22) 338.83.38

Copy for the Elected Office (EO/US)

PCT/IL00/00332

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

BEN-AMI, Paulina
Ben-Ami & Associates
P.O. Box 94
76100 Rehovot
ISRAËL

Date of mailing (day/month/year)
12 December 2001 (12.12.01)

Applicant's or agent's file reference
9956 PCT

IMPORTANT NOTIFICATION

International application No.
PCT/IL00/00332

International filing date (day/month/year)
07 June 2000 (07.06.00)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address
BEN-AMI, Paulina
Yeda Research and Development Co. Ltd.
At The Weizmann Institute of Science
P.O. Box 95
76100 Rehovot
Israel

State of Nationality:
State of Residence:
Telephone No.: 972-8-9470617
Facsimile No.: 972-8-9470739
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
BEN-AMI, Paulina
Ben-Ami & Associates
P.O. Box 94
76100 Rehovot
Israel

State of Nationality:
State of Residence:
Telephone No.: 972-8-9365090
Facsimile No.: 972-8-9365092
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Anne KARKACHI

Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

004532541

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
9956 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL00/00332	07/06/2000	07/06/1999
International Patent Classification (IPC) or national classification and IPC A61K31/00		
Applicant YEDA RESEARCH AND DEVELOPMENT CO. LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 11 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04/01/2001	25.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bochelen, D Telephone No. +49 89 2399 8150

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL00/00332

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-3,6-41	as originally filed	
4,5	with telefax of	02/09/2001

Claims, No.:

1-24	with telefax of	02/09/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:
   - ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 1, 6-8, 12-18 (all partly).

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1, 6-8, 12-18 (all partly).

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☒ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-22
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-22
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-24
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I
Basis of the report

1. The amendments filed with the fax dated 02.09.2001 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following:

   -**claim 23**: the compounds that are subject-matter of claim 23 are not disclosed in the originally filed application

   -claim 24: the disclaimer attempts to exclude of compounds of formula II that are disclosed in the prior art thereby introducing new combination in the application

2. The reformulation of the originally filed **claims 1-14** as Swiss type claims is supported in the original application (see p4 l9-5, p9 l28 and claims 16-17) and is thus allowable.

3. The newly introduced disclaimer in **claim 22** (former **claim 18**) excluding specific compounds of formula I which are disclosed in document D2, does not offend Art. 34(2)(b) PCT.

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

4. A search report was established for the compounds specifically disclosed in the examples and the general inventive concept (see ISA210). The applicant is informed that no opinion regarding novelty, inventive step and industrial applicability will be formulated in respect of subject-matter which is not covered by the search report (Rule 66(1)(e) PCT).

Re Item IV
Lack of unity of invention

5. The International Examining Authority found multiple groups of invention:

The different inventions (group of) are:

1. **Claims 1-2 (partly), 3-7, 14-22**: pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound having general formula I.

2. **Claims 1-2 (partly), 8-13, 23-24**: pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound having general formula II.

The applicant payed the fee for the examination of an additional invention. Consequently, an international preliminary report is established with regard to all the claims.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive st p or industrial applicability; citations and explanations supporting such statem nt

Reference is made to the following documents:

D1: KAHANA N ET AL: 'A CONCEPTUAL APPROACH TO THE SYNTHESIS OF BIFUNCTIONAL EDTA ANALOGSEDTA-EXTENDED POLYAMIDES' JOURNAL OF ORGANIC CHEMISTRY,US,AMERICAN CHEMICAL SOCIETY. EASTON, vol. 59, no. 17, 26 August 1994 (1994-08-26), pages 4832-4837, XP000576114 ISSN: 0022-3263

D2: WARSHAWSKY A.: 'Bifunctional Chelating Agents Part 3.' J. CHEM. SOC. PERKIN TRANS. I, vol. 10, 1989, page 1781-6 XP002155815

D8: WARSHAWSKY A ET AL: 'Cytotoxicity effects of transition-metal chelators of the 5-substituted 2-hydroxyacetophenones and their oximes.' EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, vol. 30, no. 7-8, 1995, pages 553-560, XP002163945 ISSN: 0223-5234

D9: DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KIRIENKO, G. K. ET AL: 'Derivatives of 8-hydroxyquinoline as possible anthelmintics, nematocides, and fungicides' retrieved from STN Database accession no. 70:106350 HCA XP002163950 & IZV. AKAD. NAUK MOLD. SSR, SER. BIOL. KHIM. NAUKI (1967), NO. 10,

55-62 FROM: REF. ZH., KHIM. 1968, ABSTR. NO. 15N536.,

D10: JP 63 238060 A (MARUHO KK) 4 October 1988 (1988-10-04)

D11: WARNER V D ET AL: 'Quantitative structure -activity relationships for 5-substituted 8- hydroxyquinolines as inhibitors of dental plaque.' JOURNAL OF MEDICINAL CHEMISTRY, (1977 JAN) 20 (1) 92-6. , XP002163946

D12: WARNER V D ET AL: 'Synthesis and in vitro evaluation of 8-hydroxyquinoline analogs as inhibitors of dental plaque.' JOURNAL OF MEDICINAL CHEMISTRY, (1976 JAN) 19 (1) 167-9. , XP002163947

D13: BURCKHALTER, JOSEPH H. ET AL: 'Amino - and chloromethylation of 8-quinolinol -mechanism of preponderant ortho substitution in phenols under Mannich conditions' JOURNAL OF ORGANIC CHEMISTRY, vol. 26, October 1961 (1961-10), pages 4078-4083, XP002163948

D14: MATSUMURA, KONOMU ET AL: 'Condensation of chloral hydrate with 8-quinolinol' JOURNAL OF THE AMERICAL CHEMICAL SOCIETY, 1955, pages 6671-6674, XP002163949

6. **Novelty and inventive step (Article 33 (1) (2) and (3) PCT)**:

6.1 **Invention I**:

Document D1 and D2 disclose compounds that fall in the scope of **claims 1-5** (D1: p4835 scheme3; D2: p1782 scheme 3). The subject-matter of **claim 22** is delimited from the compounds that are disclosed in documents D1-D2. The prior art neither discloses nor suggests the use of **specifically disclosed compounds** of formula I for the manufacture of pharmaceutical compositions and the use thereof for the treatment of neurodegenerative diseases, e.g. stroke or Parkinson's disease. Consequently, the subject-matter of **claims 1-7, 14-22** appears to be new and involves an inventive step.

6.2 **Invention 2**:

The prior art discloses compounds that fall in the scope of formula II and pharmaceutical compositions thereof (D8: p556 tablel; D9: abstract; D10: abstract; D11: p92 col1, p95 table V; D12: p68 table I; D13: p4082 col1 §4-4; D14: p6671

col1 §3). However, the use of the **specifically disclosed compounds** falling in the scope of formula II for the treatment of neurodegenerative diseases, e.g. stroke or Parkinson's disease, are neither disclosed nor suggested in the prior art. Consequently, it is considered that the subject-matter of **claims 1-2, 8-13, 10-14 and 19** is new and involves an inventive step.

Re Item VIII

Certain observations on the international application

7. **Claim 1** does not meet the requirements of Article 6 PCT. The compounds that fall in the scope of formula I are not clearly defined since it is not clear which substituting groups fall under the scope of the terms *hydrophobic radical.*

8. **Claims 1, 6-8, 12-18** are not supported by the description as required by Article 6 PCT, as their scope is broader than justified by the description. The reasons therefor are the following: the description provides evidence of the biological activity only for a very limited number of compounds of formula I and formula II whereas the general formulas I and II encompass an extremely large number of compounds.

9. The embodiments of the invention described on pages 39-40 (compounds 18 and 22) do not fall within the scope of the claims, i.e. formula II. Compounds 18 and 22 are listed in Appendix A without any distinctive sign allowing to differentiate these "illustrative" compounds from those intended to be in the scope of the invention. This ambiguity leads to a doubt concerning the matter for which protection is sought, thereby rendering the claims unclear (Article 6 PCT).

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

BEN-AMI, Paulina
YEDA RESEARCH & DEVELOPMENT
CO. LTD
Weizmann Institute of Science
P.O. Box 95
76100 Rehovot
ISRAEL

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	25.09.2001

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
9956 PCT	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL00/00332	07/06/2000	07/06/1999

Applicant
YEDA RESEARCH AND DEVELOPMENT CO. LTD. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

COMPUTER
DATE 7 Nov 2001
BY Jane

Name and mailing address of the IPEA/
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer
Ferro Vasconcelos, M
Tel.+49 89 2399- 8062

COURTESY COPY OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT WITH ANNEXES CONTAINING NEW PAGES 4, 5 AND 5A, OF THE SPECIFICATION TO REPLACE ORIGINAL PAGES 4 AND 5 OF THE SPECIFICATION AND NEW CLAIMS 1-24 TO REPLACE ORIGINAL CLAIMS 1-19 FOR EXAMINATION IN THIS CASE

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
9956 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL00/00332	07/06/2000	07/06/1999
International Patent Classification (IPC) or national classification and IPC A61K31/00		
Applicant YEDA RESEARCH AND DEVELOPMENT CO. LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 11 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04/01/2001	25.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bochelen, D Telephone No. +49 89 2399 8150

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL00/00332

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-3,6-41	as originally filed	
4,5	with telefax of	02/09/2001

Claims, No.:

1-24	with telefax of	02/09/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:
   - ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 1, 6-8, 12-18 (all partly).

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1, 6-8, 12-18 (all partly).

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☒ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-22
	No:	Claims
Inventive step (IS)	Yes:	Claims 1-22
	No:	Claims
Industrial applicability (IA)	Yes:	Claims 1-24
	No:	Claims

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I
Basis of the report

1. The amendments filed with the fax dated 02.09.2001 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following:

   -claim 23: the compounds that are subject-matter of claim 23 are not disclosed in the originally filed application

   -claim 24: the disclaimer attempts to exclude of compounds of formula II that are disclosed in the prior art thereby introducing new combination in the application

2. The reformulation of the originally filed **claims 1-14** as Swiss type claims is supported in the original application (see p4 l9-5, p9 l28 and claims 16-17) and is thus allowable.

3. The newly introduced disclaimer in **claim 22** (former **claim 18**) excluding specific compounds of formula I which are disclosed in document D2, does not offend Art. 34(2)(b) PCT.

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

4. A search report was established for the compounds specifically disclosed in the examples and the general inventive concept (see ISA210). The applicant is informed that no opinion regarding novelty, inventive step and industrial applicability will be formulated in respect of subject-matter which is not covered by the search report (Rule 66(1)(e) PCT).

Re Item IV
Lack of unity of invention

5. The International Examining Authority found multiple groups of invention:

The different inventions (group of) are:

1. **Claims 1-2 (partly), 3-7, 14-22**: pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound having general formula I.

2. **Claims 1-2 (partly), 8-13, 23-24**: pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound having general formula II.

The applicant payed the fee for the examination of an additional invention. Consequently, an international preliminary report is established with regard to all the claims.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: KAHANA N ET AL: 'A CONCEPTUAL APPROACH TO THE SYNTHESIS OF BIFUNCTIONAL EDTA ANALOGSEDTA-EXTENDED POLYAMIDES' JOURNAL OF ORGANIC CHEMISTRY,US,AMERICAN CHEMICAL SOCIETY. EASTON, vol. 59, no. 17, 26 August 1994 (1994-08-26), pages 4832-4837, XP000576114 ISSN: 0022-3263

D2: WARSHAWSKY A.: 'Bifunctional Chelating Agents Part 3.' J. CHEM. SOC. PERKIN TRANS. I, vol. 10, 1989, page 1781-6 XP002155815

D8: WARSHAWSKY A ET AL: 'Cytotoxicity effects of transition-metal chelators of the 5-substituted 2-hydroxyacetophenones and their oximes.' EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, vol. 30, no. 7-8, 1995, pages 553-560, XP002163945 ISSN: 0223-5234

D9: DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KIRIENKO, G. K. ET AL: 'Derivatives of 8-hydroxyquinoline as possible anthelmintics, nematocides, and fungicides' retrieved from STN Database accession no. 70:106350 HCA XP002163950 & IZV. AKAD. NAUK MOLD. SSR, SER. BIOL. KHIM. NAUKI (1967), NO. 10,

55-62 FROM: REF. ZH., KHIM. 1968, ABSTR. NO. 15N536.,

D10: JP 63 238060 A (MARUHO KK) 4 October 1988 (1988-10-04)

D11: WARNER V D ET AL: 'Quantitative structure -activity relationships for 5-substituted 8- hydroxyquinolines as inhibitors of dental plaque.' JOURNAL OF MEDICINAL CHEMISTRY, (1977 JAN) 20 (1) 92-6. , XP002163946

D12: WARNER V D ET AL: 'Synthesis and in vitro evaluation of 8-hydroxyquinoline analogs as inhibitors of dental plaque.' JOURNAL OF MEDICINAL CHEMISTRY, (1976 JAN) 19 (1) 167-9. , XP002163947

D13: BURCKHALTER, JOSEPH H. ET AL: 'Amino - and chloromethylation of 8-quinolinol -mechanism of preponderant ortho substitution in phenols under Mannich conditions' JOURNAL OF ORGANIC CHEMISTRY, vol. 26, October 1961 (1961-10), pages 4078-4083, XP002163948

D14: MATSUMURA, KONOMU ET AL: 'Condensation of chloral hydrate with 8-quinolinol' JOURNAL OF THE AMERICAL CHEMICAL SOCIETY, 1955, pages 6671-6674, XP002163949

6. **Novelty and inventive step (Article 33 (1) (2) and (3) PCT):**

6.1 **Invention I**:

Document D1 and D2 disclose compounds that fall in the scope of **claims 1-5** (D1: p4835 scheme3; D2: p1782 scheme 3). The subject-matter of **claim 22** is delimited from the compounds that are disclosed in documents D1-D2. The prior art neither discloses nor suggests the use of **specifically disclosed compounds** of formula I for the manufacture of pharmaceutical compositions and the use thereof for the treatment of neurodegenerative diseases, e.g. stroke or Parkinson's disease. Consequently, the subject-matter of **claims 1-7, 14-22** appears to be new and involves an inventive step.

6.2 **Invention 2**:

The prior art discloses compounds that fall in the scope of formula II and pharmaceutical compositions thereof (D8: p556 tableI; D9: abstract; D10: abstract; D11: p92 col1, p95 table V; D12: p68 table I; D13: p4082 col1 §4-4; D14: p6671

col1 §3). However, the use of the **specifically disclosed compounds** falling in the scope of formula II for the treatment of neurodegenerative diseases, e.g. stroke or Parkinson's disease, are neither disclosed nor suggested in the prior art. Consequently, it is considered that the subject-matter of **claims 1-2, 8-13, 10-14 and 19** is new and involves an inventive step.

Re Item VIII

Certain observations on the international application

7. **Claim 1** does not meet the requirements of Article 6 PCT. The compounds that fall in the scope of formula I are not clearly defined since it is not clear which substituting groups fall under the scope of the terms *hydrophobic radical.*

8. **Claims 1, 6-8, 12-18** are not supported by the description as required by Article 6 PCT, as their scope is broader than justified by the description. The reasons therefor are the following: the description provides evidence of the biological activity only for a very limited number of compounds of formula I and formula II whereas the general formulas I and II encompass an extremely large number of compounds.

9. The embodiments of the invention described on pages 39-40 (compounds 18 and 22) do not fall within the scope of the claims, i.e. formula II. Compounds 18 and 22 are listed in Appendix A without any distinctive sign allowing to differentiate these "illustrative" compounds from those intended to be in the scope of the invention. This ambiguity leads to a doubt concerning the matter for which protection is sought, thereby rendering the claims unclear (Article 6 PCT).

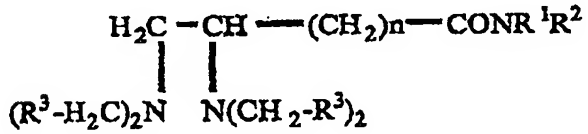
For the treatment of Parkinson's disease and probably other metal-associated neurological disorders and for the treatment of trauma and stroke and the secondary injuries which follow them, it would be highly desirable to find neuroselective iron chelators that cross the blood brain barrier.

SUMMARY OF THE INVENTION

It has now been found in accordance with the present invention that certain iron chelators which can cross the brain blood barrier are able to protect rats from neurodegenerative processes, thus making them suitable candidates for treatment of Parkinson's disease and other metal-associated neurological disorders and for treatment of trauma and stroke.

The present invention relates to the use of a compound selected from the group consisting of:

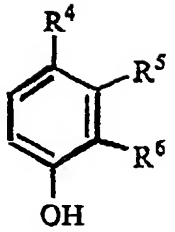
(a) a compound of formula I:



wherein

R^1 is H or hydrocarbyl; R^2 is a hydrophobic radical; R^3 is a radical selected from 3-(C_2-C_6)acyl-4-hydroxyphenyl, 3-hydroxyimino(C_2-C_6)alkyl-4-hydroxyphenyl, or COOZ, wherein Z is H, (C_1-C_6)alkyl, aryl or ar(C_1-C_6)alkyl; and n is an integer from 1 to 20; and

(b) a compound of formula II:



wherein

R^4 is (C_1-C_6)acyl, nitro(C_1-C_6)alkyl, cyano(C_1-C_6)alkyl, (C_1-C_6)alkoxy(C_1-C_6)alkyl or $-CH_2NR^7R^8$, wherein R^7 and R^8, the same or different, is each H or (C_1-C_6)alkyl, or together with the N atom form a saturated or unsaturated 5-7 membered ring optionally containing a further heteroatom selected from N, O or S, the further N atom in such saturated 5-7 membered ring being optionally substituted by C_1-C_6 alkyl, C_1-C_6 acyl, hydroxy-(C_1-C_6)alkyl, (C_1-C_6)alkoxycarbonyl, and 8-hydroxyquinolin-5-yl-(C_1-C_6)alkyl,

and

either R^5 is H and R^6 is (C_2-C_6) acyl or hydroxyimino(C_2-C_6)alkyl, or R^5 and R^6 together with the phenyl ring form a quinoline, a 1,2,3,4-tetrahydroquinoline or a perhydroquinoline ring,

or

a pharmaceutically acceptable salt thereof, for the preparation of a pharmaceutical composition for prevention of lipid peroxidation in the brain of mammals and thus for treatment of neurodegenerative disorders, particularly Parkinson's disease.

In another embodiment, the invention relates to the use of compounds of formulas I and II above for the preparation of a pharmaceutical composition for treatment of stroke.

The present invention further provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of formula I or a pharmaceutically acceptable salt thereof. These compositions are for example useful for

prevention of lipid peroxidation in the brain of mammals and thus for the treatment of neurodegenerative disorders such as for treatment of Parkinson's disease, and for treatment of stroke.

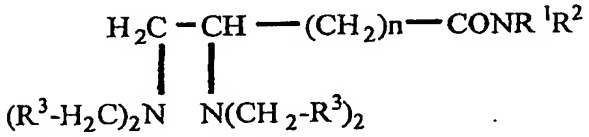
The invention further relates to novel compounds of formula I excepting the compounds N-[5-(tert-butoxycarbonyl)pentyl]-4,5-bis[(di(benzyloxycarbonyl) methyl]amino]valeramide, N-(benzyloxy-carbonylaminopropyl)-4,5-bis[(di(methoxycarbonylmethyl)amino]valeramide, N-(benzyloxycarbonylaminopropyl)-4,5-bis[[di(benzyloxy-carbonylmethyl)amino]valeramide, and N-(benzyloxy-carbonylaminoethyl)-4,5-bis[(di(carboxymethyl)amino] valeramide; to novel compounds of formula II wherein R^5 is H and R^6 is (C_2-C_6) acyl or hydroxyimino(C_2-C_6)alkyl, excepting the compounds 2-hydroxy-5-(dipropylaminomethyl)acetophenone and 2-hydroxy-5-(dipropylaminomethyl)acetophenone oxime; and to novel compounds of formula II 1 wherein R^5 and R^6 together with the phenyl ring form a quinoline, a 1,2,3,4-tetrahydroquinoline or a perhydroquinoline ring, excluding the quinoline compounds wherein R^4 is (C_1-C_2)acyl, cyanomethyl, (C_1-C_6)alkoxymethyl or $-CH_2NR^7R^8$, wherein R^7 and R^8 are both H or (C_1-C_6)alkyl, or together with the N atom form a saturated ring selected from pyrrolidino, piperidino, morpholino, and piperazino.

AMENDED SHEET

CLAIMS

1. Use of a compound selected from the group consisting of:

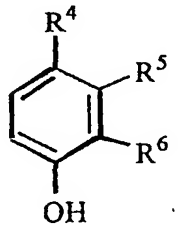
(a) a compound of formula I:



wherein

R^1 is H or hydrocarbyl; R^2 is a hydrophobic radical; R^3 is a radical selected from 3-(C_2-C_6)acyl-4-hydroxyphenyl, 3-hydroxyimino(C_2-C_6)alkyl-4-hydroxyphenyl, or COOZ, wherein Z is H, (C_1-C_6)alkyl, aryl or ar(C_1-C_6)alkyl; and n is an integer from 1 to 20; and

(b) a compound of formula II:



wherein

R^4 is (C_1-C_6)acyl, nitro(C_1-C_6)alkyl, cyano(C_1-C_6)alkyl, (C_1-C_6)alkoxy(C_1-C_6)alkyl or $-CH_2NR^7R^8$, wherein R^7 and R^8, the same or different, is each H or (C_1-C_6)alkyl, or together with the N atom form a saturated or unsaturated 5-7 membered ring optionally containing a further heteroatom selected from N, O or S, the further N atom in such saturated 5-7

membered ring being optionally substituted by C_1-C_6 alkyl, C_1-C_6 acyl, hydroxy-(C_1-C_6)alkyl, (C_1-C_6)alkoxycarbonyl, and 8-hydroxyquinolin-5-yl-(C_1-C_6)alkyl,

and

either R^5 is H and R^6 is (C_2-C_6) acyl or hydroxyimino(C_2-C_6)alkyl, or R^5 and R^6 together with the phenyl ring form a quinoline, a 1,2,3,4-tetrahydroquinoline or a perhydroquinoline ring,

or a pharmaceutically acceptable salt thereof, for the preparation of a pharmaceutical composition for prevention of lipid peroxidation in the brain of mammals and thus for treatment of neurodegenerative disorders.

2. Use according to claim 1, for the preparation of a pharmaceutical composition for treatment of Parkinson's disease.

3. Use of a compound of formula I or formula II as defined in claim 1 or a pharmaceutically acceptable salt thereof, for the preparation of a pharmaceutical composition for the treatment of stroke.

4. Use according to any one of claims 1 to 3 of a compound of formula I wherein n is 2 to 4, preferably 2; R^1 is H or a saturated, unsaturated or aromatic hydrocarbyl radical, preferably selected from C_1-C_8 alkyl, C_2-C_8 alkenyl and phenyl; R^2 is a hydrophobic radical selected from C_6-C_{20} alkyl, C_6-C_{20} alkenyl, a radical selected from C_5-C_{20} acyl, benzyloxycarbonyl, substituted benzyloxycarbonyl, C_3-C_8 alkoxycarbonyl, cycloalkoxy- carbonyl and aryloxycarbonyl, said radical being either linked directly to the N atom or through a (C_1-C_5) alkylene chain, and N-substituted amino or 4-substituted-piperazino linked to the N atom through a (C_1-C_5) alkylene chain; and R^3 is a radical selected from 3-(C_2-

C_6)acyl-4-hydroxyphenyl, 3-hydroxyimino(C_2-C_6)alkyl-4-hydroxyphenyl, or COOZ, wherein Z is H, (C_1-C_6)alkyl, aryl or ar(C_1-C_6)alkyl.

5. Use according to claim 4, wherein R^2 is straight or branched C_6-C_{20} alkyl or alkenyl; saturated or unsaturated C_5-C_{20} carboxylic acyl linked directly to the N atom or through a (C_1-C_5) alkylene chain; benzyloxycarbonyl or halo-substituted benzyloxycarbonyl, such as o- and p-chloro-benzyloxycarbonyl, 2,4- and 2,6-dichlorobenzyloxycarbonyl, linked directly to the N atom or through a (C_1-C_5) alkylene chain; a bulky alkoxycarbonyl group such as tert-butoxycarbonyl linked directly to the N atom or through a (C_1-C_5) alkylene chain; cycloalkoxycarbonyl linked directly to the N atom or through a (C_1-C_5) alkylene chain; aryloxycarbonyl such as fluorenylmethoxycarbonyl, linked directly to the N atom or through a (C_1-C_5) alkylene chain; 4-substituted-piperazinyl or N-substituted amino, linked to the N atom through a (C_1-C_5) alkylene chain, wherein the 4- and N-substituent is a hydrophobic group selected from C_6-C_{20} alkyl, C_6-C_{20} alkenyl, C_5-C_{20} acyl, benzyloxycarbonyl, substituted benzyloxycarbonyl, C_3-C_8 alkoxycarbonyl, cycloalkoxycarbonyl, aryloxycarbonyl, N-substituted amino and 4-substituted-piperazinyl, all such substituents being as defined above.

6. Use according to claim 5, wherein n is 2, R^1 is H, R^2 is a radical $-(CH_2)_3NHCOOCH_2C_6H_5$, 5-(tert-butoxycarbonyl)pentyl, or $-(CH_2)_2$-(4-carbobenzoxy)-piperazinyl, and R^3 is benzyloxycarbonyl, 3-(1-hydroxy-iminoethyl)-4-hydroxyphenyl or 3-acetyl-4-hydroxyphenyl.

7. Use according to claim 6, of a compound of formula I selected from:

N-[2-(4-carbobenzoxypiperazin-1-yl)ethyl]-4,5- bis[bis (benzyloxycarbonylmethyl)amino]valeramide (1)

N-(3-benzyloxycarbonylaminopropyl)-4,5-bis[bis (3-acetyl-4-hydroxybenzyl)amino]valeramide (2)

N-(3-benzyloxycarbonylaminopropyl)-4,5-bis[bis(3-(1-hydroxy-iminoethyl)-4-hydroxybenzyl)amino]valeramide (3)

N-[5-(tert-butyloxycarbonyl)pentyl]-4,5-bis[(bis (benzyloxycarbonyl)methyl]amino]valeramide (4)

8. Use according to any one of claims 1 to 3, of a compound of formula II wherein R^4 is C_1-C_6 acyl, nitro(C_1-C_6)alkyl in which the (C_1-C_6)alkyl group may be branched, cyano(C_1-C_6)alkyl, preferably cyanomethyl, (C_1-C_6) alkoxy(C_1-C_6)alkyl, preferably methoxymethyl, or $CH_2NR^7R^8$, in which R^7 and R^8 are both H, or one is H and the other is (C_1-C_6) alkyl, or both R^7 and R^8 are C_1-C_6 alkyl, or R^7 and R^8 together with the N-atom form a saturated or unsaturated 5-7 membered ring optionally containing a further heteroatom selected from N, O or S, the further N-atom in such saturated 5-7 membered ring being optionally substituted by (C_1-C_6) alkyl, (C_1-C_6) acyl, hydroxy-(C_1-C_6)alkyl, (C_1-C_6) alkoxycarbonyl, and 8-hydroxyquinolin-5-yl(C_1-C_6) alkyl, preferably 8-hydroxyquinolin-5-yl-methyl.

9. Use according to claim 8, wherein R^4 is a radical selected from formyl, 2-methyl-2-nitropropyl, cyanomethyl, methoxymethyl, (diethyl)amino-methyl, piperidinomethyl, morpholinomethyl, thiomorpholinomethyl, piperazinomethyl, imidazolylmethyl, 4-methyl-piperazinomethyl, 4-(2-hydroxy-ethyl)piperazinomethyl, 4-formylpiperazinomethyl,4-(ethoxy-carbonyl)piperazinomethyl,4-(butoxycarbonyl)piperazino-methyl, 4-(8-hydroxyquinolin-5-yl-methyl)-piperazinomethyl, and 4-(8-hydroxy-quinolin-5 yl-methyl)homopiperazinomethyl.

10. Use according to claim 8 or 9, of a compound of formula II wherein R^5 is H and R^6 is (C_2-C_6) acyl, preferably acetyl, or hydroxyimino(C_2-C_6)alkyl, preferably hydroxyiminoethyl.

11. Use according to claim 10, of a compound of formula II selected from:

2-acetyl-4-[4-(2-hydroxyethyl)piperazin-1-yl-methyl] phenol (5)

2-(1-hydroxyiminoethyl)-4-[4-(2-hydroxyethyl)piperazin -1-ylmethyl]phenol (6)

12. Use according to claim 8 or 9, of a compound of formula II wherein R^5 and R^6 together with the phenyl ring form a quinoline ring.

13. Use according to claim 12, of a quinoline compound selected from:

5-formyl-8-hydroxyquinoline (7)
5-(2-methyl-2-nitropropyl)-8-hydroxyquinoline (9)
5-methoxymethyl-8-hydroxyquinoline (10)
5-diethylaminomethyl-8-hydroxyquinoline (11)
5-piperidinomethyl-8-hydroxyquinoline (12)
5-morpholinomethyl-8-hydroxyquinoline (13)
5-(4-methylpiperazinomethyl)-8-hydroxyquinoline (14)
5-[4-(2-hydroxyethyl)piperazinomethyl]-8-hydroxy-quinoline (15)
5-[4-ethoxycarbonylpiperazinomethyl)-8-hydroxy-quinoline (16)
5-(imidazol-1-ylmethyl)-8-hydroxyquinolin (17)
5-(4-Boc-piperazinomethyl)-8-hydroxyquinoline (19)
5-piperazinomethyl-8-hydroxyquinoline (20)
N.N'-di-(8-hydroxyquinolin-5-ylmethyl) piperazine (21)
5-(4-formylpiperazinomethyl)-8-hydroxyquinoline (23)
5-cyanomethyl-8-hydroxyquinoline (24)

N.N'-di-(8-hydroxyquinolin-5-ylmethyl)homopiperazine, and

5-thiomorpholinylmethyl-8-hydroxyquinoline (26)

14. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of formula I in claim 1 or a pharmaceutically acceptable salt thereof.

15. A pharmaceutical composition according to claim 14 for prevention of lipid peroxidation in the brain of mammals and thus for the treatment of neurodegenerative disorders..

16. A pharmaceutical composition according to claim 15 for treatment of Parkinson's disease.

17. A pharmaceutical composition according to claim 14 for treatment of stroke.

18. A pharmaceutical composition according to any one of claims claim 14 to 17, comprising a compound of formula I wherein n is 2 to 4, preferably 2; R^1 is H or a saturated, unsaturated or aromatic hydrocarbyl radical, preferably selected from C_1-C_8 alkyl, C_2-C_8 alkenyl and phenyl; R^2 is a hydrophobic radical selected from C_6-C_{20} alkyl, C_6-C_{20} alkenyl, a radical selected from C_5-C_{20} acyl, benzyloxycarbonyl, substituted benzyloxycarbonyl, C_3-C_8 alkoxycarbonyl, cycloalkoxy- carbonyl and aryloxycarbonyl, said radical being either linked directly to the N atom or through a (C_1-C_5) alkylene chain, and N-substituted amino or 4-substituted-piperazino linked to the N atom through a (C_1-C_5) alkylene chain; and R^3 is a radical selected from 3-(C_2-C_6)acyl-4-hydroxyphenyl, 3-hydroxyimino(C_2-C_6)alkyl-4-

hydroxyphenyl, or COOZ, wherein Z is H, (C_1-C_6)alkyl, aryl or ar(C_1-C_6)alkyl.

19. A pharmaceutical composition according to claim 18, wherein R^2 is straight or branched C_6-C_{20} alkyl or alkenyl; saturated or unsaturated C_5-C_{20} carboxylic acyl linked directly to the N atom or through a (C_1-C_5) alkylene chain; benzyloxycarbonyl or halo-substituted benzyloxycarbonyl, such as o- and p-chloro-benzyloxycarbonyl, 2,4- and 2,6-dichlorobenzyloxycarbonyl, linked directly to the N atom or through a (C_1-C_5) alkylene chain; a bulky alkoxycarbonyl group such as tert-butoxycarbonyl linked directly to the N atom or through a (C_1-C_5) alkylene chain; cycloalkoxycarbonyl linked directly to the N atom or through a (C_1-C_5) alkylene chain; aryloxycarbonyl such as fluorenylmethoxycarbonyl, linked directly to the N atom or through a (C_1-C_5) alkylene chain; 4-substituted-piperazinyl or N-substituted amino, linked to the N atom through a (C_1-C_5) alkylene chain, wherein the 4- and N-substituent is a hydrophobic group selected from C_6-C_{20} alkyl, C_6-C_{20} alkenyl, C_5-C_{20} acyl, benzyloxycarbonyl, substituted benzyloxycarbonyl, C_3-C_8 alkoxycarbonyl, cycloalkoxycarbonyl, aryloxycarbonyl, N-substituted amino and 4-substituted-piperazinyl, all such substituents being as defined above.

20. A pharmaceutical composition according to claim 19, wherein n is 2, R^1 is H, R^2 is a radical $-(CH_2)_3NHCOOCH_2C_6H_5$, 5-(tert-butoxycarbonyl)pentyl, or $-(CH_2)_2$-(4-carbobenzoxy)-piperazinyl, and R^3 is benzyloxycarbonyl, 3-(1-hydroxy-iminoethyl)-4-hydroxyphenyl or 3-acetyl-4-hydroxyphenyl.

21. A pharmaceutical composition according to claim 20, comprising a compound of formula I selected from:

N-[2-(4-carbobenzoxypiperazin-1-yl)ethyl]-4,5- bis[bis (benzyloxycarbonylmethyl)amino]valeramide (1)

N-(3-benzyloxycarbonylaminopropyl)-4,5-bis[bis(3-acetyl -4-hydroxybenzyl)amino]valeramide (2)

N-(3-benzyloxycarbonylaminopropyl)-4,5-bis[bis(3-(1-hydroxy-iminoethyl)-4-hydroxybenzyl)amino]valeramide (3)

N-[5-(tert-butyloxycarbonyl)pentyl]-4,5-bis[(bis (benzyloxycarbonyl)methyl]amino]valeramide (4)

22. A compound of formula I in claim 1, excepting the compounds N-[5-(tert-butoxycarbonyl)pentyl]-4,5-bis[(di (benzyloxycarbonyl)methyl]amino]valeramide, N-(benzyloxy-carbonylaminopropyl)-4,5-bis[(di(methoxycarbonylmethyl) amino]valeramide, N-(benzyloxycarbonylaminopropyl)-4,5-bis[[di(benzyloxycarbonylmethyl) amino]valeramide, and N-(benzyloxycarbonylaminoethyl)-4,5-bis[(di(carboxymethyl) amino]valeramide.

23. A compound of formula II in claim 1 wherein R^5 is H and R^6 is (C_2-C_6) acyl or hydroxyimino(C_2-C_6)alkyl, excepting the compounds 2-hydroxy-5-(dipropylaminomethyl)acetophenone and 2-hydroxy-5-(dipropylaminomethyl)acetophenone oxime.

24. A compound of formula II in claim 1 wherein R^5 and R^6 together with the phenyl ring form a quinoline, a 1,2,3,4-tetrahydroquinoline or a perhydroquinoline ring, excluding the quinoline compounds wherein R^4 is (C_1-C_2)acyl, cyanomethyl, (C_1-C_6)alkoxymethyl or $-CH_2NR^7R^8$, wherein R^7 and R^8 are both H or (C_1-C_6)alkyl, or together with the N atom form a saturated ring selected from pyrrolidino, piperidino, morpholino, and piperazino.

PATENT COOPERATION TREATY

PCT

US.

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
9956 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IL 00/00332	07/06/2000	07/06/1999
Applicant		
YEDA RESEARCH AND DEVELOPMENT CO. LTD. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 8 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   NOVEL IRON CHELATORS AND PHARMACEUTICAL COMPOSITIONS COMPRISING IRON CHELATORS FOR THE TREATMENT OF NEURODEGENERATIVE DISORDERS

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. I, II

   [X] as suggested by the applicant.

   [ ] None of the figures.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 00/00332

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: Claims 1,12-17, (partial); 2 - 5, 18 (complete).

   Pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound having the formula I shown in claim 1.

2. Claims: Claims 1, 12-17, (partial); 6-11, 19 (complete).

   Pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound having the formula II shown in claim 1.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1,6,7,8,12-18 relate to an extremely large number of possible compounds/compositions. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds/compositions claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds explicitally disclosed at page 37-41 of the application, with due regard to the general idea underlying the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/ 00/00332

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/165 A61K31/137 A61K31/15 A61K31/47 A61K31/4709
A61P25/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBASE, MEDLINE, BIOSIS, CHEM ABS Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KAHANA N ET AL: "A CONCEPTUAL APPROACH TO THE SYNTHESIS OF BIFUNCTIONAL EDTA ANALOGSEDTA-EXTENDED POLYAMIDES" JOURNAL OF ORGANIC CHEMISTRY,US,AMERICAN CHEMICAL SOCIETY. EASTON, vol. 59, no. 17, 26 August 1994 (1994-08-26), pages 4832-4837, XP000576114 ISSN: 0022-3263 * See scheme 3, compounds N. 5 * ---	1-5, 12-14
Y	WARSHAWSKY A.: "Bifunctional Chelating Agents Part 3." J. CHEM. SOC. PERKIN TRANS. I, vol. 10, 1989, page 1781-6 XP002155815 * See compounds in figure at page 1783 * ---	1-5, 12-14

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 April 2001	

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Veronese, A

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 329 481 A (NEORX CORP) 23 August 1989 (1989-08-23) claims; figures 2A,2B,3A,3B, ---	1-5, 12-14
A	HALL E D ET AL: "Neuroprotective efficacy of microvascularly-localized versus brain-penetrating antioxidants." ACTA NEUROCHIRURGICA. SUPPLEMENTUM, (1996) 66 107-13. REF: 23, XP000972206 figure 1; table 1 ---	1-5, 12-17
A	US 4 652 519 A (WARSHAWSKY ABRAHAM ET AL) 24 March 1987 (1987-03-24) the whole document ---	1-5, 12-14
A	WESEMANN, W. (1) ET AL: "Effect of lazaroid U-74389G on iron -induced reduction of striatal dopamine metabolism." JOURNAL OF NEURAL TRANSMISSION SUPPLEMENT, (1995) VOL. 46, NO. 0, PP. 175-182., XP000972216 cited in the application the whole document ---	1-5, 12-17
A	BEN-SHACHAR D ET AL: "IRON MELANIN INTERACTION AND LIPID PEROXIDATION IMPLICATIONS FOR PARKINSON'S DISEASE." J NEUROCHEM, (1991) 57 (5), 1609-1614., XP000972207 the whole document ---	1-5, 12-17
X	WARSHAWSKY A ET AL: "Cytotoxicity effects of transition-metal chelators of the 5-substituted 2-hydroxyacetophenones and their oximes." EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, vol. 30, no. 7-8, 1995, pages 553-560, XP002163945 ISSN: 0223-5234 tables 1,2 --- -/--	1,6-8, 12-14,19

8

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KIRIENKO, G. K. ET AL: "Derivatives of 8-hydroxyquinoline as possible anthelmintics, nematocides, and fungicides" retrieved from STN Database accession no. 70:106350 HCA XP002163950 abstract & IZV. AKAD. NAUK MOLD. SSR, SER. BIOL. KHIM. NAUKI (1967), NO. 10, 55-62 FROM: REF. ZH., KHIM. 1968, ABSTR. NO. 15N536.,	1,6,7, 10-14,19
X	JP 63 238060 A (MARUHO KK) 4 October 1988 (1988-10-04) abstract	1,6,7, 10-14,19
X	WARNER V D ET AL: "Quantitative structure -activity relationships for 5-substituted 8- hydroxyquinolines as inhibitors of dental plaque." JOURNAL OF MEDICINAL CHEMISTRY, (1977 JAN) 20 (1) 92-6. , XP002163946 table 1	1,6,7, 10-14
X	WARNER V D ET AL: "Synthesis and in vitro evaluation of 8- hydroxyquinoline analogs as inhibitors of dental plaque." JOURNAL OF MEDICINAL CHEMISTRY, (1976 JAN) 19 (1) 167-9. , XP002163947 figures; tables	1,6,7, 10-14,19
X	BURCKHALTER, JOSEPH H. ET AL: "Amino - and chloromethylation of 8- quinolinol -mechanism of preponderant ortho substitution in phenols under Mannich conditions" JOURNAL OF ORGANIC CHEMISTRY, vol. 26, October 1961 (1961-10), pages 4078-4083, XP002163948 page 4081	1,6,7, 10-14,19
X	MATSUMURA, KONOMU ET AL: "Condensation of chloral hydrate with 8- quinolinol" JOURNAL OF THE AMERICAL CHEMICAL SOCIETY, 1955, pages 6671-6674, XP002163949 the whole document	1,6,7, 10-14

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/00332

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0329481 A	23-08-1989	US 5202451 A	13-04-1993
		JP 2152956 A	12-06-1990
		US 5606028 A	25-02-1997
US 4652519 A	24-03-1987	NONE	
JP 63238060 A	04-10-1988	NONE	

COURTESY COPY OF THE PCT APPLICATION AS ORIGINALLY FILED WITH ABSTRACT

Replaced by A.A. 34 Amendment.

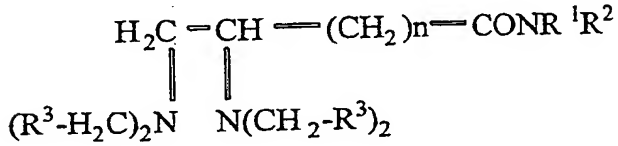
For the treatment of Parkinson's disease and probably other metal-associated neurological disorders and for the treatment of trauma and stroke and the secondary injuries which follow them, it would be highly desirable to find neuroselective iron chelators that cross the blood brain barrier.

SUMMARY OF THE INVENTION

It has now been found in accordance with the present invention that certain iron chelators which can cross the brain blood barrier are able to protect rats from neurodegenerative processes, thus making them suitable candidates for treatment of Parkinson's disease and other metal-associated neurological disorders and for treatment of trauma and stroke.

The present invention relates to pharmaceutical compositions comprising a pharmaceutically acceptable carrier and as active ingredient a compound selected form the group consisting of:

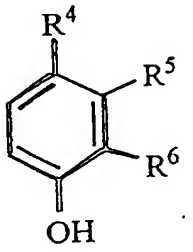
(a) a compound of formula I:



wherein

R^1 is H or hydrocarbyl; R^2 is a hydrophobic radical; R^3 is a radical selected from 3-(C_2-C_6)acyl-4-hydroxyphenyl, 3-hydroxyimino(C_2-C_6)alkyl-4-hydroxyphenyl, or COOZ, wherein Z is H, (C_1-C_6)alkyl, aryl or ar(C_1-C_6)alkyl; and n is an integer from 1 to 20; and

(b) a compound of formula II:



wherein

R^4 is (C_1-C_6)acyl, nitro(C_1-C_6)alkyl, cyano(C_1-C_6)alkyl, (C_1-C_6)alkoxy(C_1-C_6)alkyl or $-CH_2NR^7R^8$, wherein R^7 and R^8, the same or different, is each H or (C_1-C_6)alkyl, or together with the N atom form a saturated or unsaturated 5-7 membered ring optionally containing a further heteroatom selected from N, O or S, the further N atom in such saturated 5-7 membered ring being optionally substituted by C_1-C_6 alkyl, C_1-C_6 acyl, hydroxy-(C_1-C_6)alkyl, (C_1-C_6)alkoxycarbonyl, and 8-hydroxyquinolin-5-yl-(C_1-C_6)alkyl,

and

either R^5 is H and R^6 is (C_2-C_6) acyl or hydroxyimino(C_2-C_6)alkyl, or R^5 and R^6 together with the phenyl ring form a quinoline, a 1,2,3,4-tetrahydroquinoline or a perhydroquinoline ring,

and

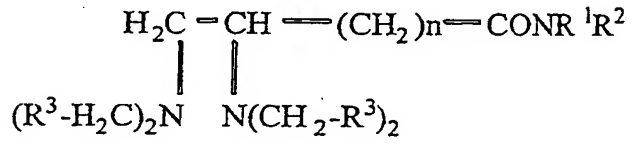
pharmaceutically acceptable salts of the compounds of formulas I and II.

The invention further relates to novel compounds of formula I, excepting the compound N-[5-(tert-butoxy-carbonyl)pentyl]-4,5-bis[(bis(benzyloxycarbonyl)methyl] amino]valeramide, and to novel compounds of formula II, excepting the compounds 5-formyl-8-hydroxyquinoline and 5-methoxymethyl-8-hydroxyquinoline.

CLAIMS

1. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound selected from the group consisting of:

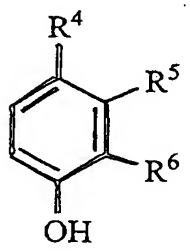
(a) a compound of formula I:



wherein

R^1 is H or hydrocarbyl; R^2 is a hydrophobic radical; R^3 is a radical selected from 3-(C_2-C_6)acyl-4-hydroxyphenyl, 3-hydroxyimino(C_2-C_6)alkyl-4-hydroxyphenyl, or COOZ, wherein Z is H, (C_1-C_6)alkyl, aryl or ar(C_1-C_6)alkyl; and n is an integer from 1 to 20; and

(b) a compound of formula II:



wherein

R^4 is (C_1-C_6)acyl, nitro(C_1-C_6)alkyl, cyano(C_1-C_6)alkyl, (C_1-C_6)alkoxy(C_1-C_6)alkyl or $-CH_2NR^7R^8$, wherein R^7 and R^8, the same or different, is each H or (C_1-C_6)alkyl, or together with the N atom form a saturated or unsaturated 5-7 membered ring optionally containing a further heteroatom selected

from N, O or S, the further N atom in such saturated 5-7 membered ring being optionally substituted by C_1-C_6 alkyl, C_1-C_6 acyl, hydroxy-(C_1-C_6)alkyl, (C_1-C_6)alkoxycarbonyl, and 8-hydroxyquinolin-5-yl-(C_1-C_6)alkyl,

and

either R^5 is H and R^6 is (C_2-C_6) acyl or hydroxyimino(C_2-C_6)alkyl, or R^5 and R^6 together with the phenyl ring form a quinoline, a 1,2,3,4-tetrahydroquinoline or a perhydroquinoline ring,

and

pharmaceutically acceptable salts of the compounds of formulas I and II.

2. A pharmaceutical composition according to claim 1, comprising a compound of formula I wherein n is 2 to 4, preferably 2; R^1 is H or a saturated, unsaturated or aromatic hydrocarbyl radical, preferably selected from C_1-C_8 alkyl, C_2-C_8 alkenyl and phenyl; R^2 is a hydrophobic radical selected from C_6-C_{20} alkyl, C_6-C_{20} alkenyl, a radical selected from C_5-C_{20} acyl, benzyloxycarbonyl, substituted benzyloxycarbonyl, C_3-C_8 alkoxycarbonyl, cycloalkoxy-carbonyl and aryloxycarbonyl, said radical being either linked directly to the N atom or through a (C_1-C_5) alkylene chain, and N-substituted amino or 4-substituted-piperazino linked to the N atom through a (C_1-C_5) alkylene chain; and R^3 is a radical selected from 3-(C_2-C_6)acyl-4-hydroxyphenyl, 3-hydroxyimino(C_2-C_6)alkyl-4-hydroxyphenyl, or COOZ, wherein Z is H, (C_1-C_6)alkyl, aryl or ar(C_1-C_6)alkyl.

3. A pharmaceutical composition according to claim 2, wherein R^2 is straight or branched C_6-C_{20} alkyl or alkenyl; saturated or unsaturated C_5-C_{20} carboxylic acyl linked directly to the N atom or through a (C_1-C_5) alkylene chain; benzyloxycarbonyl or halo-substituted benzyloxycarbonyl,

such as o- and p-chloro-benzyloxycarbonyl, 2,4- and 2,6-dichlorobenzyloxycarbonyl, linked directly to the N atom or through a (C_1-C_5) alkylene chain; a bulky alkoxycarbonyl group such as tert-butoxycarbonyl linked directly to the N atom or through a (C_1-C_5) alkylene chain; cycloalkoxycarbonyl linked directly to the N atom or through a (C_1-C_5) alkylene chain; aryloxycarbonyl such as fluorenylmethoxycarbonyl, linked directly to the N atom or through a (C_1-C_5) alkylene chain; 4-substituted-piperazinyl or N-substituted amino, linked to the N atom through a (C_1-C_5) alkylene chain, wherein the 4- and N-substituent is a hydrophobic group selected from C_6-C_{20} alkyl, C_6-C_{20} alkenyl, C_5-C_{20} acyl, benzyloxycarbonyl, substituted benzyloxycarbonyl, C_3-C_8 alkoxycarbonyl, cycloalkoxycarbonyl, aryloxycarbonyl, N-substituted amino and 4-substituted-piperazinyl, all such substituents being as defined above.

4. A pharmaceutical composition according to claim 3, wherein n is 2, R^1 is H, R^2 is a radical $-(CH_2)_3NHCOOCH_2C_6H_5$, 5-(tert-butoxycarbonyl)pentyl, or $-(CH_2)_2$-(4-carbobenzoxy)-piperazinyl, and R^3 is benzyloxycarbonyl, 3-(1-hydroxy-iminoethyl)-4-hydroxyphenyl or 3-acetyl-4-hydroxyphenyl.

5. A pharmaceutical composition according to claim 4, comprising a compound of formula I selected from:

N-[2-(4-carbobenzoxypiperazin-1-yl)ethyl]-4,5- bis[bis (benzyloxycarbonylmethyl)amino]valeramide (1)

N-(3-benzyloxycarbonylaminopropyl)-4,5-bis[bis (3-acetyl-4-hydroxybenzyl)amino]valeramide (2)

N-(3-benzyloxycarbonylaminopropyl)-4,5-bis[bis(3-(1-hydroxy-iminoethyl)-4-hydroxybenzyl)amino]valeramide (3)

N-[5-(tert-butyloxycarbonyl)pentyl]-4,5-bis[(bis (benzyloxycarbonyl)methyl]amino]valeramide (4)

6. A pharmaceutical composition according to claim 1, comprising a compound of formula II wherein R^4 is C_1-C_6 acyl, nitro(C_1-C_6)alkyl in which the (C_1-C_6)alkyl group may be branched, cyano(C_1-C_6)alkyl, preferably cyanomethyl, (C_1-C_6) alkoxy(C_1-C_6)alkyl, preferably methoxymethyl, or $CH_2NR^7R^8$, in which R^7 and R^8 are both H, or one is H and the other is (C_1-C_6) alkyl, or both R^7 and R^8 are C_1-C_6 alkyl, or R^7 and R^8 together with the N-atom form a saturated or unsaturated 5-7 membered ring optionally containing a further heteroatom selected from N, O or S, the further N-atom in such saturated 5-7 membered ring being optionally substituted by (C_1-C_6) alkyl, (C_1-C_6) acyl, hydroxy-(C_1-C_6)alkyl, (C_1-C_6) alkoxycarbonyl, and 8-hydroxyquinolin-5-yl(C_1-C_6) alkyl, preferably 8-hydroxyquinolin-5-yl-methyl.

7. A pharmaceutical composition according to claim 6, wherein R^4 is a radical selected from formyl, 2-methyl-2-nitropropyl, cyanomethyl, methoxymethyl, (diethyl)amino-methyl, piperidinomethyl, morpholinomethyl, thiomorpholino-methyl, piperazinomethyl, imidazolylmethyl, 4-methyl-piperazinomethyl, 4-(2-hydroxyethyl)piperazinomethyl, 4-formylpiperazinomethyl, 4-(ethoxycarbonyl)piperazinomethyl, 4-(butoxycarbonyl)piperazinomethyl, 4-(8-hydroxyquinolin-5-yl-methyl)-piperazinomethyl, and 4-(8-hydroxy-quinolin-5-yl-methyl)homopiperazinomethyl.

8. A pharmaceutical composition according to claim 6 or 7, comprising a compound of formula II wherein R^5 is H and R^6 is (C_2-C_6) acyl, preferably acetyl, or hydroxyimino(C_2-C_6)alkyl, preferably hydroxyiminoethyl.

9. A pharmaceutical composition according to claim 8, comprising a compound of formula II selected from:

2-acetyl-4-[4-(2-hydroxyethyl)piperazin-1-yl-methyl] phenol (5)

2-(1-hydroxyiminoethyl)-4-[4-(2-hydroxyethyl)piperazin -1-ylmethyl]phenol (6)

10. A pharmaceutical composition according to claim 6 or 7, comprising a compound of formula II wherein R^5 and R^6 together with the phenyl ring form a quinoline ring.

11. A pharmaceutical composition according to claim 10, comprising a quinoline compound selected from:

5-formyl-8-hydroxyquinoline (7)

5-(2-methyl-2-nitropropyl)-8-hydroxyquinoline (9)

5-methoxymethyl-8-hydroxyquinoline (10)

5-diethylaminomethyl-8-hydroxyquinoline (11)

5-piperidinomethyl-8-hydroxyquinoline (12)

5-morpholinomethyl-8-hydroxyquinoline (13)

5-(4-methylpiperazinomethyl)-8-hydroxyquinoline (14)

5-[4-(2-hydroxyethyl)piperazinomethyl]-8-hydroxy-quinoline (15)

5-[4-ethoxycarbonylpiperazinomethyl)-8-hydroxy-quinoline (16)

5-(imidazol-1-ylmethyl)-8-hydroxyquinolin (17)

5-(4-Boc-piperazinomethyl)-8-hydroxyquinoline (19)

5-piperazinomethyl-8-hydroxyquinoline (20)

N.N'-di-(8-hydroxyquinolin-5-ylmethyl) piperazine (21)

5-(4-formylpiperazinomethyl)-8-hydroxyquinoline (23)

5-cyanomethyl-8-hydroxyquinoline (24)

N.N'-di-(8-hydroxyquinolin-5-ylmethyl)homopiperazine, and

5-thiomorpholinylmethyl-8-hydroxyquinoline (26)

12. A pharmaceutical composition according to any one of claims 1 to 11 for prevention of lipid peroxidation in the brain of mammals.

13. A pharmaceutical composition according to any one of claims 1 to 12 for the treatment of stroke.

14. A pharmaceutical composition according to any one of claims 1 to 12 for the treatment of Parkinson's disease.

15. Use of a compound of formula I or formula II according to any one of claims 1 to 11 for the preparation of a pharmaceutical composition for prevention of lipid peroxidation in the brain of mammals.

16. Use of a compound of formula I or formula II according to any one of claims 1 to 11 for the preparation of a pharmaceutical composition for the treatment of stroke.

17. Use of a compound of formula I or formula II according to any one of claims 1 to 11 for the preparation of a pharmaceutical composition for the treatment of Parkinson's disease.

18. A compound of formula I in claim 1, excepting the compound N-[5-(tert-butoxycarbonyl)pentyl]-4,5-bis[(bis (benzyloxycarbonyl)methyl]amino]valeramide.

19. A compound of formula II in claim 1, excepting the compounds 5-formyl-8-hydroxyquinoline and 5-methoxymethyl-8-hydroxyquinoline.

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(19) World Intellectual Property Organization
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(22) International Filing Date: 7 June 2000 (07.06.2000)

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(71) Applicants *(for all designated States except US)*: **YEDA RESEARCH AND DEVELOPMENT CO. LTD.** [IL/IL]; Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL). **TECHNION RESEARCH AND DEVELOPMENT FOUNDATION LTD.** [IL/IL]; Gutwirth Science Park, Technion City, 32000 Haifa (IL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **WARSHAWSKY, Abraham** [IL/IL]; 8 Neve Matz, Weizmann Institute of Science, 76100 Rehovot (IL). **YOUDIM, Moussa, B., H.** [IL/IL]; 18 Hankin Street, 32763 Haifa (IL). **BEN-SHACHAR, Dorit** [IL/IL]; 60a Harishonim Street, 26302 Kiryat Haim (IL).

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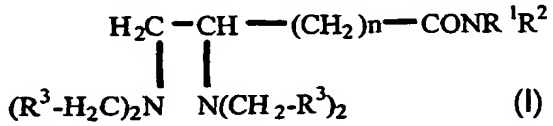
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

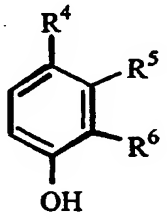
Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING IRON CHELATORS FOR THE TREATMENT OF NEURODEGENERATIVE DISORDERS AND SOME NOVEL IRON CHELATORS



(I)



(II)

(57) Abstract: Use of a compound of formula (I), wherein R^1 is H or hydrocarbyl; R^2 is a hydrophobic radical; R^3 is 3-(C_2-C_6)acyl-4-hydroxyphenyl, 3-hydroxyimino(C_2-C_6)-alkyl-4-hydroxyphenyl, or COOZ, wherein Z is H, (C_1-C_6)alkyl, aryl or ar(C_1-C_6)alkyl; and n is 1-20; and of a compound of formula (II), wherein R^4 is (C_1-C_6)acyl, nitro(C_1-C_6)alkyl, cyano(C_1-C_6)alkyl, (C_1-C_6)alkoxy(C_1-C_6)alkyl or $-CH_2NR^7R^8$, wherein R^7 and R^8, the same or different, is each H or (C_1-C_6)alkyl, or together with the N atom form a saturated or unsaturated 5-7 membered ring optionally containing a further heteroatom selected from N, O or S, the further N atom being optionally substituted, and either R^5 is H and R^6 is (C_2-C_6) acyl or hydroxyimino(C_2-C_6)alkyl, or R^5 and R^6 together with the phenyl ring form a quinoline, a 1,2,3,4-tetrahydroquinoline or a perhydroquinoline ring, for the preparation of pharmaceutical compositions for the treatment of Parkinson's disease or stroke.

WO 00/74664 A2

PHARMACEUTICAL COMPOSITIONS COMPRISING IRON CHELATORS FOR THE TREATMENT OF NEURODEGENERATIVE DISORDERS AND SOME NOVEL IRON CHELATORS

FIELD OF THE INVENTION

The present invention relates to pharmaceutical compositions comprising as active ingredients compounds that act as neuroprotective iron chelators and are suitable for the treatment of neurodegenerative disorders such as Parkinson's disease, Alzheimer-type dementia and stroke. The invention further relates to certain novel iron chelators of the type described in the specification.

BACKGROUND OF THE INVENTION

Parkinson's disease is a progressive neurodegeneration of the melanized dopaminergic neurons in the substantia nigra. It is clinically characterized mainly by akinesia, bradykinesia and tremor at rest. Postmortem studies on brains from parkinsonian patients suggest the involvement of oxygen free radical-induced oxidative stress which results in lipid peroxidation of cell membranes, followed by increased membrane fluidity and finally cell death.

Normally dopamine (DA) is metabolized by either monoamine oxidase or by autooxidation. Both ways lead to an excess of toxic oxygen species, such as H_2O_2, which in the presence of a transient metal, such as iron, will produce cytotoxic oxygen free radicals, e.g. superoxide and hydroxyl free radicals. The brain, like all other tissues, protects itself against the deleterious effects of oxygen free radicals by specific protective enzymes such as glutathione peroxidase, catalase and superoxide dismutase, and by relatively high amounts of glutathione and ascorbate. In addition, iron is bound to high molecular weight proteins

such as ferritin, hemosiderin and transferrin, or to low molecular weight molecules such as ADP, ATP, catechol and probably also melanin, and its amount in the brain is strictly conserved by the blood brain barrier (BBB).

In Parkinson's disease, the brain defensive mechanisms against the formation of cytotoxic oxygen free radicals are defective. In the substantia nigra of parkinsonian brains there are reductions in activities of superoxide dismutase and glutathione peroxidase and reduced tissue contents of glutathione and ascorbate. Moreover, iron concentrations are significantly elevated in parkinsonian substantia nigra pars compacta within the melanized dopamine neurons. These conditions favor liberation of free cytotoxic radicals, which can cause among other things release of intracellular calcium and lipid peroxidation resulting in neuronal death. Indeed an increase in basal lipid peroxidation in the substantia nigra of parkinsonian patients has been detected.

Iron alone or iron decompartmentalized from its binding site by a neurotoxin, e.g. the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA), may induce oxidative stress and neurodegeneration, as evidenced in previous studies of the inventors in which intranigral administration of iron induced "Parkinsonism" in rats and the iron chelator desferrioxamine protected the rats against 6-OHDA-induced lesions of nigrostrial dopamine neurons (D. Ben-Shachar and M.B.H. Youdim, 1991, J. Neurochem. 56: 1441-4). It has thus been suggested that treatment or retardation of the process of dopaminergic neurodegeneration in the substantia nigra may be affected by iron chelators capable of crossing the blood brain barrier in a fashion similar to chelators used in the treatment of Wilson's disease and iron overload in systemic organs.

This may be a new therapeutic approach for the treatment of Parkinson's disease that can be applied to

other metal-associated neurological disorders such as tardive dyskinesia, Alzheimer's and Hallervorden-Spatz diseases.

Stroke is the third leading cause of death in the western world today, exceeded only by heart diseases and cancer. The overall prevalence of the disease is 0.5-0.8% of the population. Stroke is characterized by a sudden appearance of neurological disorders such as paralysis of limbs, speech and memory disorders, sight and hearing defects, etc., which result from a cerebrovascular damage.

Haemorrhage and ischemia are the two major causes of stroke. The impairment of normal blood supply to the brain is associated with a rapid damage to normal cell metabolism including impaired respiration and energy metabolism lactacidosis, impaired cellular calcium homeostasis release of excitatory neurotransmitters, elevated oxidative stress, formation of free radicals, etc. Ultimately these events lead to cerebral cell death and neurological disfunction.

Treatment of stroke is primarily surgical. Much effort is being invested in less aggressive therapeutical intervention in the search for drugs which are capable of restoring normal blood perfusion in the damaged area as well as drugs which are designed to overcome the above listed damaging events associated with cellular damage.

Oxidative stress and free radical formation play a major role in tissue injury and cell death. These processes are catalyzed by transient metal ions, mainly iron and copper. In the case of stroke, since vascular damage is involved, iron is available for the free radical formation, a process that could be prevented by iron chelators. Indeed, with lazaroides (21-amino steroids), known free radical scavengers, a significant improvement of local and global ischemia damages induced in animals has been achieved.

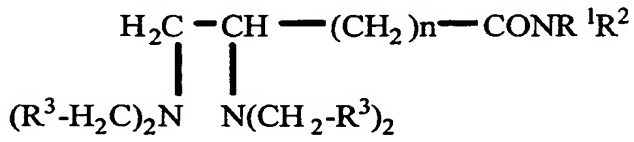
For the treatment of Parkinson's disease and probably other metal-associated neurological disorders and for the treatment of trauma and stroke and the secondary injuries which follow them, it would be highly desirable to find neuroselective iron chelators that cross the blood brain barrier.

SUMMARY OF THE INVENTION

It has now been found in accordance with the present invention that certain iron chelators which can cross the brain blood barrier are able to protect rats from neurodegenerative processes, thus making them suitable candidates for treatment of Parkinson's disease and other metal-associated neurological disorders and for treatment of trauma and stroke.

The present invention relates to pharmaceutical compositions comprising a pharmaceutically acceptable carrier and as active ingredient a compound selected form the group consisting of:

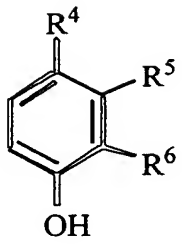
(a) a compound of formula I:



wherein

R^1 is H or hydrocarbyl; R^2 is a hydrophobic radical; R^3 is a radical selected from 3-(C_2-C_6)acyl-4-hydroxyphenyl, 3-hydroxyimino(C_2-C_6)alkyl-4-hydroxyphenyl, or COOZ, wherein Z is H, (C_1-C_6)alkyl, aryl or ar(C_1-C_6)alkyl; and n is an integer from 1 to 20; and

(b) a compound of formula II:



wherein

R^4 is (C_1-C_6)acyl, nitro(C_1-C_6)alkyl, cyano(C_1-C_6)alkyl, (C_1-C_6)alkoxy(C_1-C_6)alkyl or $-CH_2NR^7R^8$, wherein R^7 and R^8, the same or different, is each H or (C_1-C_6)alkyl, or together with the N atom form a saturated or unsaturated 5-7 membered ring optionally containing a further heteroatom selected from N, O or S, the further N atom in such saturated 5-7 membered ring being optionally substituted by C_1-C_6 alkyl, C_1-C_6 acyl, hydroxy-(C_1-C_6)alkyl, (C_1-C_6)alkoxycarbonyl, and 8-hydroxyquinolin-5-yl-(C_1-C_6)alkyl,

and

either R^5 is H and R^6 is (C_2-C_6) acyl or hydroxyimino(C_2-C_6)alkyl, or R^5 and R^6 together with the phenyl ring form a quinoline, a 1,2,3,4-tetrahydroquinoline or a perhydroquinoline ring,

and

pharmaceutically acceptable salts of the compounds of formulas I and II.

The invention further relates to novel compounds of formula I, excepting the compound N-[5-(tert-butoxy-carbonyl)pentyl]-4,5-bis[(bis(benzyloxycarbonyl)methyl] amino]valeramide, and to novel compounds of formula II, excepting the compounds 5-formyl-8-hydroxyquinoline and 5-methoxymethyl-8-hydroxyquinoline.

In the compounds of formula I, n is preferably 2 to 4, most preferably 2, in which case the compounds are derivatives of valeramide. The term "hydrocarbyl", as used herein for the radical R^1, refers to hydrocarbyl radicals that are saturated, unsaturated or aromatic, including, but not being limited to, C_1-C_8 alkyl, e.g. methyl, ethyl, propyl and butyl, C_2-C_8 alkenyl, e.g. vinyl and allyl, and phenyl.

The term "hydrophobic" radical, as used herein for R^2, includes, but is not limited to, radicals such as C_6-C_{20} alkyl; C_6-C_{20} alkenyl; a radical selected from C_5-C_{20} acyl, benzyloxycarbonyl, substituted benzyloxycarbonyl, C_3-C_8 alkoxycarbonyl, cycloalkoxycarbonyl,and aryloxycarbonyl, said radical being either linked directly to the N atom or through a (C_1-C_5) alkylene chain; and N-substituted amino or 4-substituted-piperazino linked to the N atom through a (C_1-C_5) alkylene chain.

Illustrative examples of hydrophobic radicals for R^2 include, but are not limited to, the following: C_6-C_{20} straight or branched alkyl or alkenyl such as hexyl, octyl, dodecyl, undecyl, dodecyl and the corresponding alkenyl radicals; a saturated or unsaturated C_5-C_{20} carboxylic acyl group such as, for example, an alkanoyl radical selected from hexanoyl, octanoyl, lauroyl, palmitoyl, myristoyl, stearoyl and aracidyl, and the corresponding alkenoyl radicals, linked directly to the N atom or through a (C_1-C_5) alkylene chain; benzyloxycarbonyl or halo-substituted benzyloxycarbonyl, e.g. o- and p-chloro-benzyloxycarbonyl, 2,4- and 2,6-dichlorobenzyloxycarbonyl, linked directly to the N atom or through a (C_1-C_5) alkylene chain; a bulky alkoxycarbonyl group such as tert-butoxycarbonyl (Boc), tert-amyloxycarbonyl, isopropoxycarbonyl, linked directly to the N atom or through a (C_1-C_5) alkylene chain, e.g. tert-butoxycarbonylpentyl; cycloalkoxycarbonyl, e.g. cyclo-pentoxycarbonyl, cyclohexyloxycarbonyl, adamantyloxycarbonyl

(Adoc), linked directly to the N atom or through a (C_1-C_5) alkylene chain; aryloxycarbonyl such as fluorenylmethoxycarbonyl, linked directly to the N atom or through a (C_1-C_5) alkylene chain; 4-substituted-piperazinyl or N-substituted amino, linked to the N atom through a (C_1-C_5) alkylene chain, wherein the 4- and N-substituent is a hydrophobic group such as C_6-C_{20} alkyl, C_6-C_{20} alkenyl, C_5-C_{20} acyl, benzyloxycarbonyl, substituted benzyloxycarbonyl, C_3-C_8 alkoxycarbonyl, cycloalkoxycarbonyl, aryloxycarbonyl, N-substituted amino and 4-substituted-piperazinyl, all such substituents being as defined above.

The radical R^3 in the compounds of formula I may be a group 3-(C_2-C_6)acyl-4-hydroxyphenyl, in which the C_2-C_6 carboxylic acyl may be acetyl, propionyl, butyryl, hexanoyl; a group 3-hydroxyimino(C_2-C_6)alkyl-4-hydroxyphenyl, in which the alkyl may be ethyl, propyl, butyl, hexyl; or a group COOZ in which Z is H, C_1-C_6 alkyl, e.g. methyl, ethyl, propyl, butyl, pentyl, and hexyl, aryl, e.g. phenyl, or aralkyl, such as benzyl.

In preferred embodiments of the invention in the compounds of formula I, n is 2, R^1 is H and R^2 is a radical $-(CH_2)_3NHCOOCH_2C_6H_5$, 5-(tert-butoxycarbonyl)pentyl, or $-(CH_2)_2$-(4-carbobenzoxy)piperazinyl, and R^3 is benzyloxy-carbonyl, 3-(1-hydroxy-iminoethyl)-4-hydroxyphenyl or 3-acetyl-4-hydroxyphenyl. Examples are the compounds of formula I identified as **Compounds 1-4** in the Appendix A just before the claims.

The compounds of formula II in which R^5 is H and R^6 is (C_2-C_6) acyl or hydroxyimino(C_2-C_6)alkyl represent keto derivatives of phenol and their corresponding oximes. The acyl is preferably C_2-C_6 saturated aliphatic acyl, such as, for example, acetyl, propionyl, butyryl, hexanoyl; and the (C_2-C_6)alkyl is for example, ethyl, propyl, butyl, pentyl.

In the compounds of formula II, R^4 may be C_1-C_6 acyl, such as, for example, formyl, acetyl, propionyl, butyryl, caproyl; nitro(C_1-C_6)alkyl, in which the (C_1-C_6)alkyl group may be branched, such as, for example, 2-methyl-2-nitropropyl; cyano(C_1-C_6)alkyl, e.g. cyanomethyl, cyano-propyl; (C_1-C_6) alkoxy(C_1-C_6)alkyl, such as, for example, methoxymethyl, ethoxymethyl; $CH_2NR^7R^8$, in which R^7 and R^8 are both H, or one is H and the other is C_1-C_6 alkyl, or both R^7 and R^8 are alkyl, such as, for example the radical $CH_2NR^7R^8$ may be aminomethyl, methylaminomethyl, ethylaminomethyl, dimethyl- aminomethyl, diethylaminomethyl, or R^7 and R^8 together with the N-atom form a saturated or unsaturated 5-7 membered ring optionally containing a further heteroatom selected from N, O or S, the further N-atom in such saturated 5-7 membered ring being optionally substituted by C_1-C_6 alkyl, e.g. methyl, ethyl, propyl, isopropyl, butyl; C_1-C_6 acyl, e.g. formyl, acetyl, propionyl; hydroxy-(C_1-C_6)alkyl, e.g. hydroxymethyl, hydroxyethyl, hydroxypropyl; (C_1-C_6)alkoxycarbonyl, e.g. methoxycarbonyl, ethoxycarbonyl, tert-butoxycarbonyl; and 8-hydroxyquinolin-5-yl(C_1-C_6)alkyl, for example, 8-hydroxyquinolin-5-yl-methyl. For example, R^4 as a radical $CH_2NR^7R^8$ may be piperidinomethyl, morpholinomethyl, thiomorpholinomethyl, piperazinomethyl, 4-methylpiperazinomethyl, 4-(2-hydroxyethyl)piperazino-methyl, 4-formylpiperazinomethyl, 4-(ethoxycarbonyl)-piperazinomethyl, 4-(butoxycarbonyl)piperazinomethyl, 4-(8-hydroxyquinolin-5-yl-methyl)-piperazinomethyl, 4-(8-hydroxy-quinolin-5-yl- methyl)homopiperazinomethyl, and imidazolylmethyl.

In a preferred embodiment, the compounds of formula II are phenol derivatives as represented by the **Compounds 5 and 6** in the Appendix A just before the claims.

In another preferred embodiment, the compounds of formula II are 8-hydroxyquinoline derivatives as represented

by the **Compounds 7, 9-17, 19-21, 23-26** in the Appendix A just before the claims, preferably the **Compound 15.**

The compounds of the invention are prepared by chemical synthesis methods well known in the art. Some of these methods are illustrated herein in the Examples. For the preparation of other compounds of formulas I and II, similar procedures known to those of skill in the art may be used.

The compounds of formulas I and II were found according to the present invention to prevent lipid peroxidation in brain homogenates *in vitro*.

The present invention thus provides pharmaceutical compositions, useful to prevent lipid peroxidation in the brain of mammals comprising a compound of formula I or II herein or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier.

The pharmaceutically acceptable salts according to the invention may be salts formed with compounds of formula I wherein R^3 is COOH or are addition salts formed by reaction with inorganic acids such as hydrochloric, hydrobromic, sulfuric or phosphoric acids, or with organic acids such as acetic, propionic, maleic, fumaric, benzoic, citric, tartaric, or oxalic acids, by methods well-known in the art.

In another aspect, the present invention provides the use of a compound of formula I or II herein or of a pharmaceutically acceptable salt thereof as neuroprotective iron chelators for the preparation of pharmaceutical compositions to prevent lipid peroxidation in the brain of mammals and, thus, for the treatment of neurodegenerative diseases such as Parkinson's disease, and for the treatment of stroke.

In still another aspect, the invention relates to a method for the treatment of neurodegenerative diseases such as Parkinson's disease, or for the treatment of stroke, which comprises administering to an individual in need

thereof an effective amount of a compound of formula I or of formula II or of a pharmaceutically acceptable salt thereof.

DETAILED DESCRIPTION OF THE INVENTION

The iron chelator compounds I and II of the pharmaceutical compositions of the invention are useful for the treatment of Parkinson's disease and probably other metal-associated neurological disorders and for the treatment of trauma and stroke and the secondary injuries which follow them, by virtue of their ability to cross the blood brain barrier and to prevent lipid peroxidation in the brain, a process which leads to neuronal death.

The ability of the compounds of the invention to prevent lipid peroxidation in brain tissue was first screened in rat brain homogenates *in vitro* by a method involving the detection of free radicals performed by metabolism of thiobarbituric acid (TBA) to malondialdehyde (MDA) and measurement of the MDA formation, as described by D. Ben-Shachar et al. (1991) J. Neurochem. 57: 1609-14. In this method, brain cortex homogenates are prepared in sucrose and incubated alone to determine basal lipid peroxidation, or incubated after the addition of $Fe_2(SO_4)_3$ or $FeCl_3$ for Fe-induction of maximum free-radical formation, and in the presence of the iron chelators to be tested. After addition of TBA, lipid peroxidation is assayed by measurement of MDA formation.

The ability of iron chelators to act as neuroprotectors was first demonstrated in an animal model of Parkinson's disease (intraventricular injection of 6-hydroxydopamine (6-OHDA)) using the iron chelator desferrioxamine (D. Ben-Shachar et al. (1991) J. Neurochem. 56: 1441-44). A selective increase in content of iron in the pars compacta of the substantia nigra has been implicated in the biochemical pathology of Parkinson's disease. Iron is

thought to induce oxidative stress by liberation of oxygen free radicals from H_2O_2. Because 6-OHDA is thought to induce nigrostriatal dopaminergic neuronal lesions via metal-catalyzed free radical formation, the effect of the iron chelator desferrioxamine was investigated on 6-OHDA-induced dopaminergic neuron degeneration in the rat. Intracerebroventricular injection of 6-OHDA (250 µg) caused a 88, 79 and 70% reduction in striatal tissue content of dopamine (DA), 3-4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), respectively and a 2.5-fold increase in DA release as indicated by the HVA/DA ratio. Prior injection of desferrioxamine (130 ng and 13 ng, i.c.v.) resulted in a significant protection (~60% and 100%, respectively) against the 6-OHDA-induced reduction in striatal DA content and a normalization of DA release Dopaminergic-related behavioral responses, such as spontaneous movements in a novel environment and rearing, were significantly impaired in the 6-OHDA-treated group. By contrast, the desferrioxamine-pretreated rats exhibited almost normal behavioral responses. The ability of iron chelators to retard dopaminergic neurodegeneration in the substantia nigra indicates a new therapeutic strategy in the treatment of Parkinson's disease.

According to the present invention, compounds of formulas I and II were injected to rats as described in D. Ben-Shachar et al. (1991) J. Neurochem. 56: 1441-44 and were shown to efficiently prevent the 6-OHDA-induced reduction in striatal dopamine and DOPAC concentrations in the rat.

For preparing the pharmaceutical compositions of the present invention, methods well-known in the art can be used. Inert pharmaceutically acceptable carriers can be used that are either solid of liquid. Solid form preparations include powders, tablets, dispersible granules, capsules, cachets and suppositories.

A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, or tablet disintegrating agents; it can also be an encapsulating material.

Liquid pharmaceutical compositions include solutions, suspensions, and emulsions. As an example, water or water-propylene glycol solutions for parenteral injection may be mentioned. Liquid preparations can also be formulated in solution in aqueous polyethylene glycol solution. Aqueous solutions for oral use can be prepared by dissolving the active component in water and adding suitable colorants, flavoring agents, stabilizers, and thickening agents as desired. Aqueous suspensions for oral use can be made by dispersing the finely divided active component in water with viscous material, i.e., natural or synthetic gums, resins, methyl cellulose, sodium carboxymethyl cellulose, and other well-known suspending agents.

Preferably, the pharmaceutical composition is in unit dosage form. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, for example, packeted tablets, capsules, and powders in vial or ampoules. The unit dosage form can also be a capsule, cachet, or table itself or it can be the appropriate number of any of these packaged forms.

In therapeutic use for the treatment of Parkinson's disease, the compounds utilized in the pharmaceutical method of this invention may be administered to the patient at dosage levels of from 1 mg/Kg to 20 mg/Kg per day.

In therapeutic use for the treatment of stroke one or more dosages of from about 100 mg/Kg to about 500 mg/Kg of

body weight may be administered to the patient as soon as possible after the event.

The dosage, however, may be varied depending upon the requirements of the patient, the severity of the condition being treated, and the compound being employed. Determination of optimum dosages for a particular situation is within the skill of the art.

The following examples illustrate particular methods for preparing compounds in accordance with this invention. These examples are illustrative and are not to be read as limiting the scope of the invention as it is defined by the appended claims.

EXAMPLES

The formulas of the compounds of Examples 1-26, herein designated **Compounds 1-26**, are presented in **Appendix A**, shown just before the Claims.

EXAMPLE 1

Synthesis of N-[2-(4-carbobenzoxypiperazin-1-yl)ethyl]-4,5-bis[bis(benzyloxycarbonylmethyl)amino]valeramide (1)

To a solution containing N-[2-(4-carbobenzoxypiperazin-1-yl(ethyl]-4,5-diaminovaleramide (100mg, 0.27mmol) in 1ml CH_3CN (freshly distilled over P_2O_5), a mixture of tetramethylnaphthalene-1,8-diamine (0.306g, 1.43mmol) and NaI (0.021g, 0.14mmol) in 0.12ml freshly distilled CH_3CN was added. The mixture was heated slightly and stirred under a nitrogen atmosphere to dissolve all components, benzyl 2-bromoacetate was added thereto(0.22ml, 0.328g, 1.43mmol), and the mixture was refluxed at 96°C for 22h under a nitrogen atmosphere.

Subsequently, the precipitate was filtered off and the solvent evaporated. $CHCl_3$ was then added to the filtrate, the solid filtered off once again, and the solvent evaporated. To remove excess benzyl bromoacetate, the residual oil was then washed a few times with hexane, and finally dried under vacuum to yield 300mg crude product. The product was then purified by flash chromatography, using $CHCl_3$:MeOH as the eluent. 47mg of the title product were obtained. No further purification was carried out.

EXAMPLE 2

Synthesis of N-(3-benzyloxycarbonylaminopropyl)-4,5-bis[bis (3-acetyl-4-hydroxybenzyl)amino]valeramide (2)

A suspension of 2-acetyl-4-chloromethylphenol (0.48g; 2.6mmol), N-(3-benzyloxycarbonylaminopropyl)-4,5-diamino-valeramide (0.14g; 0.43mmol), diisopropyl(ethyl)amine (0.47ml; 2.69mmol) in DMF (10ml) was stirred at room temperature for 24h. The mixture was evaporated to dryness. $CHCl_3$ (80ml) was added to the residue, the reaction mixture was filtered off and the solvent was evaporated. The oil was purified by flash chromatography on silica gel using 1% MeOH/$CHCl_3$ as the eluent to receive the pure title product (0.152mg; 38%). TLC (2% MeOH/$CHCl_3$), R_f=0.22.

EXAMPLE 3

Synthesis of N-(3-benzyloxycarbonylaminopropyl)-4,5-bis[bis (3-(1-hydroxy-iminoethyl)-4-hydroxybenzyl)amino]valeramide (3)

A suspension of **Compound 2** of Example 2 (0.55g; 0.06mmol), $NH_2OH \cdot HCl$ (0.042g; 0.6mmol) and $NaHCO_3$ (0.055g; 0.065mmol) in MeOH (15ml) was stirred at 65°C for 48h. $CHCl_3$

(50ml) was added to the reaction mixture. The precipitate was filtered off, the solvent was evaporated, and the residue was purified by flash chromatography on silica gel using $CHCl_3$ and 5% MeOH/$CHCl_3$ as the eluents. 12mg (20%) of the title product was eluted with 10% MeOH/$CHCl_3$. The product is not soluble in $CHCl_3$. TLC(10% MeOH/$CHCl_3$). R_f=0.15.

EXAMPLE 4

Synthesis of N-[5-(tert-butyloxycarbonyl)pentyl]-4,5-bis [(bis(benzyloxycarbonyl)methyl]amino]valeramide (4)

N,N,N',N'-Tetramethylnaphthalene-1,8-diamine (2.18g; 10.2 mmol) and NaI (0.15g; 1mmol) were added to a solution of N-[5-(tert-butyloxycarbonyl)pentyl]-4,5-diaminovaleramide (described in Kahana et al., (1994) J. Org. Chem., Vol. 59, 4832-37) (0.58g; 1.9mmol) in CH_3CN (freshly distilled on 3ml P_2O_5) and the reaction mixture was placed in a silicon oil bath at 95°C. Benzyl 2-bromoacetate (1.6ml; 10.2mmol) was added, and the mixture was refluxed under N_2 for 42h and then cooled to room temperature. The solid was filtered off and washed with $CHCl_3$. The filtrate and washing were evaporated, and the residual oil was washed (x3) with ethyl acetate/hexane (1:9) to remove excess benzyl bromoacetate. The solvent was decanted and the residue (2.14g, brown oil) was flash chromatographed on silica gel using 0.25% MeOH/ $CHCl_3$ as eluant to give the title product as a yellow-brown oil (o.38g, 22% yield).

EXAMPLE 5

Synthesis of 2-acetyl-4-[4-(2-hydroxyethyl)piperazin-1-yl-methyl]phenol (5)

2-Piperazin-1-yl-ethanol (260mg, 2mmol) and 2-acetyl-4-chloromethyl phenol (368mg, 2mmol) were stirred in

chloroform at room temperature. Sodium carbonate (106mg, 1mmol) was added and the reaction mixture was stirred overnight. The solid was filtered off and the organic layer washed with water followed by brine, dried over sodium sulfate, filtered and evaporated to obtain the crude product, which was crystallized from ethyl acetate-hexane to receive the title product as yellowish-white crystals (400mg 72%), mp=72-75°C. $C_{15}H_{22}N_2O_3$ requires: N 10.06 found: N 9.70.

^{1}NMR: d($CDCl_3$)=12.22 (S, 1H, Ph**OH**), 7.65 (d, 1H, J=1.99Hz, **Ph**; 7.445 (dd, 1H, J_1=8.62Hz, J_2=2.18Hz, **Ph**); 6.94 (d, 1H, J=8.48Hz, **Ph**); 3.62 (t, 2H, J=5.25Hz, **CH_2**OH); 3.46 (S, 2H, Ph**CH_2**); 2.65 (S, 3H, CO**CH_3**); 2.57-2.41 (m, 11H, **CH_2**x5+**OH**).

EXAMPLE 6

Synthesis of 2-(1-hydroxyiminoethyl)-4-[4-(2-hydroxyethyl) piperazin-1-ylmethyl]phenol (6)

Hydroxylamine hydrochloride (63mg, 0.9 mmol) and sodium bicarbonate (76mg, 0.9 mmol) were dissolved in distilled water (1ml). 2-Acetyl-4-[4-(2-hydroxyethyl)-piperazin-1-yl-methyl]phenol (85mg, 0.3 mmol) in absolute methanol (2ml) was added and the reaction mixture was stirred at 65°C for 24h. $CHCl_3$ (20ml) was then added, the organic phase washed with water followed by brine, dried over Na_2SO_4, filtered and evaporated to obtain the title product (52mg, 81%).

^{1}NMR: d ($CDCl_3$)=7.36 (d, 1H, J=1.94Hz, **Ph**); 7.15 (dd, 1H, J_1=2.0Hz, J_2=8.29Hz, **Ph**); 6.87 (d, 1H, J=8.28Hz, **Ph**); 3.65 (t, 10H, J=5.4Hz, **CH_2**x5+1H, **OH**); 2.31 (S, 3H, **CH_3**).

EXAMPLE 7

Synthesis of 5-formyl-8-hydroxyquinoline (7)

The title compound is prepared in two steps:

7.1 5-(2,2,2-trichloro-1-hydroxyethyl)-8-hydroxyquinoline **(8)**

To trichloracetaldehyde (41.6g; 0.28 mol) was added con. H_2SO_4 (1 drop) and the mixture was mixed. This chloral was decantated (without the acid) into 8-hydroxyquinoline (27.17g; 0.187 mol). The reaction was exotermic. After a few minutes of mixing, the reaction mixture was left standing for 3 days at room temperature until it turned to a light yellow solid, and then stirred at 65-70°C in silicon oil bath for 35h. After cooling, the reaction mixture was stirred with 3N HCl (470ml; 140ml 32% HCl+water --- 470ml) at 80°C for 1.5h (using mechanical stirrer) until the orange reaction mass completely turned to yellow crystalline hydrochloride, which was filtered after cooling. The crystals were suspended in hot water (375ml) and sodium acetate trihydrate (75g; 0.55 mol) was added to the suspension. The mixture was stirred on a water bath (80°C) for 30 min. The resulting orange-yellow free base was filtered after cooling and washed with hot water and dried under high vacuum with P_2O_5. Yield - 44.0g (80%) (from Bull. Chem. Soc. Jp. 42:1741 (1969).

7.2 5-Formyl-8-hydroxyquinoline **(7)**

Analytic acetone (220ml) was added to a 3-necked flask equipped with mechanical stirrer which was placed in dry ice-acetone bath, under Ar. Na (4.5g: 0.2mol) was added to the cooled acetone during 30 min, then 5-chloralyl-8-hydroxyquinoline (**Compound 8**) (12.0g; 0.041 mol) was added to the acetone suspension and the resulting mixture was stirred for 2-3h at 25°C. After standing for 3 days at room temperature, the resulting precipitate was filtered in buchner, washed with acetone and dried by air. Then the precipitate was dissolved in water (100ml) and was treated

by charcoal (2 teaspoons). After filtration, the solution was neutralized with a 50% solution of CH_3CO_2H (few drops). A straw yellow precipitate was filtered (mother solution 1) and dried in a desiccator over P_2O_5 to receive 3.2g. A mixture of this precipitate (3.2g) and sodium disulfite (10.4g; 54.7 mmol) was well stirred in water (21ml) at 60°C using magnetic stirrer (with charcoal: 2 teaspoons). After cooling, the mixture was filtered and the precipitate washed with water. Concentrated HCl (35ml) was added to the combined filtrate and washings, the solution was stirred with heating until the evolution gas SO_2 ceased, and then concentrated to get solid + solution (10ml). After standing overnight the separated solid was filtered, dissolved in hot water (70ml) and the solution was treated with charcoal and then filtered. Upon addition of $NaOAc.3H_2O$ (4.2g) to the filtrate the free base separated, which was filtered and washed with water. Yield: 1.0g. It was recrystallized from benzene to form almost colorless prisms. M.p. 177-8°C (in capillary).

EXAMPLE 8

Synthesis of 5-(2-methyl-2-nitropropyl)-8-hydroxyquinoline (9)

A solution of 2-nitropropane (30 ml, 0.33mmol) in DMF (20ml) was added to a mixture of 5-chloromethyl-8-hydroxy-quinoline hydrochloride (3g; 13mmol) and potassium tert-butoxide (5.6g, 50mmol) at 5°C under Ar atmosphere. The reaction mixture was stirred for 24h at room temperature. $CHCl_3$ (100ml) was then added, and the solution was washed with water until a neutral pH was obtained. It was then washed with brine, dried over Na_2SO_4 and evaporated to dryness under vacuum (50°C/1mm/Hg). The residue was crystallized from ethanol (50ml) yielding 1.4g (43%) of the

title product. M.p. 133-134°C; TLC ($CHCl_3$/MeOH/NH_3-8:2:0.5). R_f=0.8.

EXAMPLE 9

Synthesis of 5-methoxymethyl-8-hydroxyquinoline **(10)**

5-Chloromethyl-8-hydroxyquinoline hydrochloride (2.145 g; 9.3mmol) was added to a mixture of sodium methoxide (1.763g; 32.6 mmol) in MeOH (40ml). The reaction mixture was stirred for about 4h at room temperature, and then evaporated to dryness. The residue was dissolved in $CHCl_3$ (100ml, the solution was washed with water until a neutral pH was obtained, and was then washed with brine, dried over Na_2SO_4 and evaporated to dryness. The residue was extracted with hexane (100ml). The hexane solution was evaporated to give the title product, 0.36g (20%). M.p. 75-76°C. TLC ($CHCl_3$/MeOH/NH_3·9.5:0.5:0.1). R_f=0.36.

EXAMPLE 10

Synthesis of 5-diethylaminomethyl-8-hydroxyquinoline **(11)**

Diethylamine (2.4ml; 23.2mmol) was added to a mixture of 5-chloromethyl-8-hydroxyquinoline hydrochloride (2.131g; 9.25mmol) in $CHCl_3$ (50ml) at 5°C. The reaction mixture was stirred for 24h at room temperature. $CHCl_3$ (50ml) was then added and the solution was washed with 5% $NaHCO_3$ (2x50ml) and brine (50ml) and dried over Na_2SO_4. The solution was filtered and evaporated to dryness. The residue was crystallized from hexane (~10-15ml) and gave 1.23g (58%) of the product. An analytic sample of the title product was obtained by sublimation (80°C/1mm Hg). M.p.=71-72°C.

Example 11

Synthesis of 5-piperidinomethyl-8-hydroxyquinoline **(12)**

Piperidine (2ml; 20.26mmol) was added to a solution of 5-chloromethyl-8-hydroxyquinoline (1.87g; 8.13mmol) in $CHCl_3$ (50ml) at 5°C. The mixture was stirred for two days at room temperature. Then the mixture was evaporated under vacuum to dryness. The residue was dissolved in $CHCl_3$, washed with 5% $NaHCO_3$ (2x50ml), followed by brine (50ml), dried over Na_2SO_4 and evaporated to dryness. The residue was crystallized from hexane to give 1.0g of the title product (50%). M.p. 96°C. TLC ($CHCl_3$; MeOH; NH_3=8:2:0.5). R_f=0.63.

EXAMPLE 12

Synthesis of 5-morpholinomethyl-8-hydroxyquinoline (13)

Morpholine (1.9ml; 21.8mmol) was added to a solution of 5-chloromethyl-8-hydroxyquinoline (1.98g; 8.34mmol) in $CHCl_3$ (50ml) at 5°C. The reaction mixture was stirred overnight at room temperature. Then $CHCl_3$ (100ml) was added and the solution was washed with 5% $NaHCO_3$ (2x50ml), followed by brine (50ml), and dried over Na_2SO_4. The solution was filtered and evaporated under vacuum to dryness. The residue was crystallized from hexane-$CHCl_3$ and gave 1.2g (59%) of the title product. M.p. 130°C. TLC ($CHCl_3$; MeOH; NH_3=8:2:0.5. R_f=0.69.

EXAMPLE 13

Synthesis of 5-(4-methylpiperazinomethyl)-8-hydroxyquinoline (14)

N-methylpiperazine (5.0ml), 45mmol) was added to a mixture of 5-chloromethyl-8-hydroxyquinoline hydrochloride (4.1g; 17.8mmol) in $CHCl_3$ (80ml) at 5°C. The mixture was stirred for 24 h at room temperature. $CHCl_3$ (100ml) was then added and the solution was washed with 5% $NaHCO_3$ (3x50ml) and

brine 2x50ml) and then dried over Na_2SO_4. The solution was filtered and evaporated to dryness. The residue was crystallized from a mixture of benzene-hexane and gave 2.89 g (63%) of the title product. M.p. 126-127°C. TLC ($CHCl_3$-MeOH-NH_3 9:1:0.1) R_f=0.35.

EXAMPLE 14

Synthesis of 5-(4-(2-hydroxyethyl)piperazin-1-ylmethyl)-8-hydroxyquinoline **(15)**

4-(2-Hydroxyethyl)-piperazine (7.2ml; 58.7mmol) was added to a suspension of 5-chloromethyl-8-hydroxyquinoline (5.413g; 23.5mmol) in $CHCl_3$ (80ml) at 0°C. The mixture was stirred overnight at room temperature. The reaction mixture was subsequently washed with a saturated $NaHCO_3$ solution and brine, then dried with Na_2SO_4 and evaporated to dryness. Crystallization of the residue from a mixture of $CHCl_3$-Hex gave 4.05g (60%) of title product. M.p. 123-4°C. The mother liquor was evaporated and the residue was crystallized to yield 1.5g of title product. Overall yield: 5.55g (82%). A highly pure product was obtained by soxleth extraction using hexane as the extractant. TLC ($CHCl_3$ MeOH NH_3=8:2:0.5). R_f=0.4.

EXAMPLE 15

Synthesis of 5-(4-ethoxycarbonylpiperazinomethyl)-8-hydroxyquinoline **(16)**

N-Ethoxycarbonylpiperazine (1.5ml, 10.2mmol) was added to a mixture of 5-chloromethyl-8-hydroxyquinoline hydrochloride (2.36g, 10.2mmol) and diisopropylethyamine (3.6ml, 20.6mmol) in $CHCl_3$ (50ml) at 5°C. The mixture was stirred for 24h at room temperature. $CHCl_3$ (100ml) was then added and the solution was washed with 5% $NaHCO_3$ (3x50ml) and

brine (2x50ml) and then dried over Na_2SO_4. The solution was filtered and evaporated to dryness. The residue was crystallized from a mixture of benzene hexane and gave 1.38 g (42%) of the title product. M.p.-96°C. TLC ($CHCl_3$-MeOH-NH_3 9:1:0.1) R_f=0.6; TLC ($CHCl_3$-MeOH-Me_3 9:0.5:0.05) R_f-0.4.

EXAMPLE 16

Synthesis of 5-(imidazol-1-ylmethyl)-8-hydroxyquinoline (17)

A mixture of 5-chloromethyl-8-hydroxyquinoline hydrochloride (3.45g; 15mmol), imidazole (1.02g; 15mmol) and diisopropylethylamine (5.25ml; 30mmol) in $CHCl_3$ (60ml) was stirred for 24h at room temperature and then for 3h at 60°C. After cooling, the mixture was evaporated, washed with ethyl acetate (50ml) and then hexane (50ml). The residue was crystallized from a mixture of toluene and ethanol (abs.) to give 0.83g (29%) of title product. M.p. 182°C.

EXAMPLE 17

Synthesis of N-Boc-Piperazine (18)

A solution of di-tertbutyl dicarbonate (0.217g, 1mmol) in absolute methanol was added dropwise to piperazine (0.172g, 2mmol) in absolute methanol (10ml) during 0.5h with stirring. The reaction mixture was stirred for 2h, then the methanol was evaporated and the residue dissolved in ethylacetate (50ml) The ethyl acetate solution was then washed with distilled water (3 times, 10ml) followed by 10% citric acid (15ml) and then evaporated under vacuum at 40°C. The product was obtained as a white solid (0.175g, 94% yield), m.p. = 40-42 °C. TLC: R_f=0.61, CH_3Cl : MeOH : NH_3(aq) 9 : 1: 0.25. ^{1}H NMR-δ ($CDCl_3$) = 1.42 (9H, s, H_3)

Elemental analysis: $C_9H_{18}N_2O_2$ (M.W. 186.25)- Required: **H**-9.74; **C**-58.04; **N**-15.04. Found: **H**-9.62; **C**-58.15; **N**-14.93.

EXAMPLE 18

Synthesis of 5-(N'-Boc-piperazinomethyl)-8-hydroxyquinoline (19)

5-Chloromethyl-8-hydroxyquinoline hydrochloride (1g, 4.35mmol), N-Boc-piperazine (**Compound 18**) (0.81g, 4.35mmol) and diisopropylethylamine (1.489g, 2ml, 11.5mmol) were stirred in chloroform (30ml) at room temperature overnight. Then chloroform (20ml) was added and the reaction mixture washed with saturated sodium carbonate solution (15ml x2) followed by brine (20ml). The organic phase was separated and dried over anhydrous sodium sulfate overnight. Then the chloroform solution was evaporated under vacuum at room temperature. The product obtained was a green compound (1.36g, 91%). Crystallization from benzene yielded green crystals, m.p.=118-120°C. TLC: R_f=0.61, CH_3Cl:MeOH:NH_3(aq) 9 : 1: 0.25.

^{1}H NMR- δ **($CDCl_3$)** = 8.77 (1H, dd, J1 = 4.19 Hz, J2 = 1.54 Hz, ***H_2***); 8.65 (1H, dd, J1 = 8.55 Hz, J2 = 1.57 Hz, ***H_4***); 7.45 (1H, dd, J1 = 8.55 Hz, J2 = 4.20 Hz, ***H_3***); 7.31 (1H, d, J = 7.73 Hz, ***H_6***); 7.06 (1H, d, J = 7.72 Hz, ***H_7***); 3.80 (2H, s, ***H_5***); 3.37 (4H, s, ***H_{10}***); 2.40 (4H, s, ***H_9***); 1.43 (9H, s, ***H_{11}***)

Elemental analysis- $C_{19}H_{25}N_3O_3$ (M.W. 343.19). Required: **H**-7.34; **C**-66.44; **N**-12.24. Found: **H**-7.22; **C**-66.10; **N**-12.21.

EXAMPLE 19

Synthesis of 5-piperazinomethyl-8-hydroxyquinoline trichloride (20)

Compound **19** (1g) was dissolved in dry dioxane (30ml). 4M HCl in dioxane (20ml) was added and the reaction mixture

was stirred for 2h at room temperature. The dioxane was then removed under vacuum at 60°C to obtain the product as a yellow powder (1.1g, 100%).

Neutralization of the product: the product (0.150g) was dissolved in H_2O (25ml). $NaHCO_3$ (sat) (25ml) was added and the solution was stirred for 20 min. Then chloroform (150 ml) was added and the mixture stirred for a further 30 min. The two phases separated, the organic phase was dried over Na_2SO_4, filtered and evaporated. The white powder obtained was refluxed with benzene (50ml) using a Din-Stark apparatus, followed by reflux with pentene (50ml). After complete evaporation of pentene, the free base product was obtained as a white powder (0.76g). m.p. = 232-234°C (with decomposition.) TLC: R_f=0.28, CH_3Cl:MeOH:NH_3(aq) 9 : 1: 0.25.

^{1}H NMR

δ **($CDCl_3$)**=8.77(1H,dd,J1=4.18 Hz, J2=1.54 Hz, ***H_2***); 8.66 (1H, dd, J1 = 8.53 Hz, J2 = 1.54 Hz, ***H_4***); 7.45 (1H, dd, J1 = 8.55 Hz, J2 = 4.20 Hz, ***H_3***); 7.31 (1H, d, J = 7.73 Hz, ***H_6***); 7.05 (1H, d, J = 7.71 Hz, ***H_7***); 3.77 (2H, s, ***H_5***); 2.84 (4H, t, J = 4.87 Hz, ***H_{10}***); 2.44 (4H, not resolved triplet, ***H_9***).

Elemental analysis - $C_{14}H_{17}N_3O$ (M.W. 243.13). Required: **H**-7.00; **C**-69.14. Found: **H**-6.89; **C**-67.97.

EXAMPLE 20

Synthesis of N,N'-di-(8-hydroxyquinolin-5-ylmethyl)-piperazine tetrachloride (21)

5-Chloromethyl-8-hydroxyquinoline hydrochloride (1.5g, 3 equivalents) was added to absolute chloroform (40ml) followed by the addition of diisopropylethylamine (2.27ml, 6 equivalents) at 5°C. The reaction mixture was shaked it became clear, then piperazine (0.187g, 1 equivalent) was added and the reaction mixture was shaked 36h. The white

precipitate was filtered and dissolved in 2M hydrochloric acid (40ml) Yellow water solution was then liofilized to get 1g (84%) of yellow powder.

For the elemental analysis, NMR, and melting point measurements hydrochloric acid-free (neutral) compound was prepared. Bis-hydroxyquinoline tetrachloride (200mg) was dissolved in water (25ml), and then saturated sodium hydrocarbonate solution (25ml) was added and the mixture was shaked for 20 minutes. Then chloroform (150ml) was added. Water-chloroform mixture was shaked strongly 30 minutes and then chloroform solution was separated from water, dried overnight with anhydrous sodium sulphate and then evaporated. White powder was then boiled with benzene (50 ml) using Din-Stark attachment, and then boiled with pentene (50ml) After the complete evaporation of pentene, 93mg of white powder was obtained, m.p = 227-228 °C. TLC: R_f = 0.27, CH_3Cl : MeOH : NH_3(aq) 9 : 1: 0.25

^{1}H NMR

δ **($CDCl_3$)** = 8.76 (2H, dd, J1 = 4.20 Hz, J2 = 1.52 Hz, 2×***H*****$_2$**); 8.64 (2H, dd, J1 = 8.52 Hz, J2 = 1.28 Hz, 2×***H*****$_4$**); 7.45 (2H, dd, J1 = 8.52 Hz, J2 = 4.20 Hz, 2×***H*****$_3$**); 7.31 (2H, d, J = 7.68 Hz, 2×***H*****$_6$**); 7.05 (2H, d, J = 7.72 Hz, 2×***H*****$_7$**); 3.80 (4H, s, 4×***H*****$_5$**); 2.49 (8H, not resolved, 8×***H*****$_9$**)

Elemental analysis - $C_{24}H_{24}N_4O_2$ (M.W. 400.48). Required: **H**-6.00; **C**-72.00. Found: **H**-6.18; **C**-71.88.

EXAMPLE 21

Synthesis of N-Formylpiperazine (22)

Methylformiate (20ml, 290mmol) was added at 5°C to piperazine (25g, 290mmol) and the reaction mixture was stirred 2h at room temperature, followed by 12h at 80°C (in an oil bath while the flask was equiped with a reflux

condenser). Methanol was removed under vacuum at 50°C and then piperazine was removed by sublimation at vacuum at 100°C. (The reaction mixture was heated until condensation of piperazine was finished.) The product was obtained as colourless liquid that was condensed at ~130°C (yield: 18ml (61%), n_{20}^{d} = 1.121g/l.TLC: R_f = 0.45, CH_3Cl : MeOH : NH_3(aq) 9 : 1: 0.25.

^{1}H NMR - δ **($CDCl_3$)** = 7.99 (1H, s, ***H_4***)
Elemental analysis - $C_5H_6N_2O$ (M.W. 110.12). Required: **H**-5.49; **C**-54.54; **N**-25.44. Found: **H**-5.71; **C**-54.23; **N**-25.11.

EXAMPLE 22

Synthesis of 5-(4-formylpiperazinomethyl)-8-hydroxyquinoline (23)

5-Chloromethyl-8-hydroxyquinoline hydrochloride (2.26g, 9.8mmol) piperazine formamide (1.0g, 9mmol) and diisopropylethylamine (2.75g, 21mmol) were stirred in chloroform (30ml) for 48h. Then chloroform (150ml) was added and the reaction mixture was washed with Na_2CO_3 (25ml x2), followed by brine (20ml). The organic phase was dried over Na_2SO_4 for 8h, filtered and evaporated. The product was obtained as a green solid (2.2g, 95%) which was crystallized from benzene. m.p. = 172-174 °C. Additional purification of the product could be done by crystallization from benzene.
TLC: R_f = 0.49, CH_3Cl : MeOH : NH_3(aq) 9 : 1: 0.25
^{1}H NMR
δ **($CDCl_3$)** = 8.78 (1H, dd, J1 = 4.20 Hz, J2 = 1.56 Hz, ***H_2***); 8.62 (1H, dd, J1 = 8.55 Hz, J2 = 1.57 Hz, ***H_4***); 8.00 (1H, s, ***H_{11}***); 7.46 (1H, dd, J1 = 8.54 Hz, J2 = 4.19 Hz, ***H_3***); 7.31 (1H, d, J = 7.73 Hz, ***H_6***); 7.06 (1H,d,J=7.71 Hz, ***H_7***); 3.82 (2H, s, 2×***H_5***)

Elemental analysis - $C_{14}H_{17}N_3O$ (M.W. 243.31). Required: **H**-6.27; **C**-66.34; **N**-15.48. Found: **H**-6.31; **C**-66.11; **N**-15.41.

EXAMPLE 23

Synthesis of 5-piperazinomethyl-8-hydroxyquinoline trichloride (20) (alternative method)

A solution of ~16% HCl in methanol (25ml) was added to a solution of compound **23** (300mg, 1.23mmol) in absolute methanol (5ml). (Upon addition of the acid, all insoluble material was dissolved). The reaction mixture was stirred at room temperature. After 10 min, a yellow powder was precipitated; the mixture was stirred overnight. The product was then filtered and washed with absolute methanol (5ml x2). The product was obtained as a yellow powder in quantitative yield. TLC and the m.p. showed the product to be identical to that obtained previously.

EXAMPLE 24

Synthesis of 5-cyanomethyl-8-hydroxyquinoline (24)

5-Chloromethyl-8-hydroxyquinoline hydrochloride (2.5g, 1mmol) was dissolved in DMSO (15ml, technical grade). The solution was cooled in an ice bath and diisopropylethylamine (3ml, 16.7mmol) was added. The mixture was stirred until all starting material had dissolved. Subsequently, a solution of NaCN (2g, 40mmol) in DMSO (10ml, technical grade) was prepared in a 50ml flask and cooled in an ice bath. The hydroxyquinoline was then added dropwise during ~6 minutes. The ice bath was then removed and the reaction mixture wass stirrred for 3.5h at 45°C. The mixture was then added to an ice-cold solution of $NaHCO_3$ (sat) (50ml) and H_2O (50ml). The product precipitated during ~20 min. The mixture was then filtered and the solid was washed twice

with cold water (20ml + 30ml), and dried under high vacuum to remove traces of water. The product was obtained as a white powder (1.06g, 53%), m.p. = 171-172°C.TLC: R_f = 0.43, CH_3Cl : MeOH : NH_3(aq) 9 : 1: 0.25

^1H NMR

δ **($CDCl_3$)** = 8.77 (1H, dd, J_1 = 4.19 Hz, J_2 = 1.54 Hz, ***H2***); 8.65 (1H, dd, J_1 = 8.55 Hz, J_2 = 1.57 Hz, ***H4***); 7.45 (1H, dd, J_1 = 8.55 Hz, J_2 = 4.20 Hz, ***H3***); 7.31 (1H, d, J = 7.73 Hz, ***H6***); 7.06 (1H, d, J = 7.72 Hz, ***H7***); 3.80 (2H, s, ***H5***);

Elemental analysis - $C_{11}H_8N_2O$ (M.W. 184.20). Required: **H**-4.34; **C**-71.66; **N**-15.20. Found: **H**-4.33; **C**-71.93; **N**-14.89.

EXAMPLE 25

Synthesis of N,N'-di-(8-hydroxyquinolin-5-yl-methyl)-homopiperazine (25)

5-Chloromethyl-8-hydroxyquinoline hydrochloride (1.5g, 6.5mmol) was dissolved in abs $CHCl_3$ (40ml). Diisopropylethylamine (2.82g, 22mmol) was added. The mixture was stirred until all material had dissolved. Homopiperazine (0.2g, 2mmol) was then added, and the mixture stirred for a further 48h at room temperature. Subsequently, $CHCl_3$ (200ml) was added and the mixture was washed with $NaHCO_3$(sat) and then with water. The organic phase was dried overnight over Na_2SO_4, filtered and the solvent evaporated to yield a white powder (0.75g). The dry product was obtained by azeotropic distillation with benzene, followed by reflux with pentene and evaporation, yielding a white powder (0.7g, 65%). m.p = 155-157 °C.

TLC: R_f = 0.32, CH_3Cl : MeOH : NH_3(aq) 9 : 1: 0.25

^1H NMR

δ **($CDCl_3$)** = 8.76 (2H, dd, J_1 = 4.16 Hz, J_2 = 1.53 Hz, 2×***H2***); 8.68 (2H, dd, J_1 = 8.53 Hz, J_2 = 1.45 Hz, 2×***H4***); 7.43 (2H,

dd, J_1 = 8.54 Hz, J_2 = 4.21 Hz, 2×***H3***); 7.25 (2H, d, J = 3.49 Hz, 2×***H6***); 7.03 (2H, d, J = 7.71 Hz, 2×***H7***); 3.88 (4H, s, 4×***H5***); 2.72 (4H, t, J = 5.89, 4×***H9***); 2.61 (4H, s, 4×***H11***); 1.75 (2H, t, J = 5.56, 2×***H10***)

Elemental analysis - $C_{25}H_{26}N_4O_2$ (M.W. 414.51). Required: **H**-6.28; **C**-72.46; **N**-13.53. Found:**H**-6.10; **C**-73.13; **N**-12.97.

EXAMPLE 26

Synthesis of 5-thiomorpholinomethyl-8-hydroxyquinoline (26)

Thiomorpholine (1ml; 10mM) was added to a solution of 5-chloromethyl-8-quinolinol hydrochloride (2.3g; 10mM) and DIEA (3.5ml; 20.1mM) in chloroform (50ml) at 5°C. The reaction mixture was stirred for 24h at room temperature. 50ml of chloroform was then added and the solution was washed twice with 50ml of 5% sodium hydrocarbonate solution. Then the chloroform solution was filtered and evaporated to dryness. The residue was then crystallized from hexane-$CHCl_2$ and gave 1.5g (58%) of the product, m.p. = 121-122 °C

TLC: R_f = 0.39, CH_3Cl : MeOH : NH_3(aq) 9 : 1: 0.25

^{1}H NMR

δ **($CDCl_3$)** = 8.78 (1H, dd, J1 = 4.17 Hz, J2 = 1.56 Hz, ***H2***); 8.64 (1H, dd, J1 = 8.52 Hz, J2 = 1.55 Hz, ***H4***); 7.45 (1H, dd, J1 = 8.56 Hz, J2 = 4.21 Hz, ***H3***); 7.31 (1H, d, J = 7.73 Hz, ***H6***); 7.07 (1H, d, J = 7.72 Hz, ***H7***); 3.80 (1H, s, ***H5***)

Elemental analysis - $C_{14}H_{16}N_2S$ (M.W. 260.35). Required: **N**-10.76; **S**-12.31. Found: **N**-10.59; **S**-12.19.

EXAMPLE 27

Prevention of lipid peroxidation in brain tissue

Brain cortex homogenates (10% wt/vol) from male Wistar rats were prepared in 0.3M sucrose and incubated in air as described (Rehncrona et al., (1980) J. Neurochem. 34:

1630-38). Aliquots (0.1ml) of homogenate were incubated alone at 30°C for 90 min to determine basal lipid peroxidation, or incubated after the addition of 10^{-4} $Fe_2(SO_4)_o$ or $FeCl_3$ and in the presence of 10^{-3}M iron chelator of formula I or II. For the assay, to 0.3ml of the homogenate there were added 0.2ml of 8% SDS, 1.5ml of 20% acetic acid pH 3.0-3.5, 1.5ml of 0.8% thiobarbituric acid (TBA) and 0.5ml of H_2O_2 x2, the mixture was incubated at 95°C for 60 min, cooled and lipid peroxidation was assayed by measurement of malondialdehyde formation at 532nm, as described (Dexter et al. (1989) J. Neurobiochem. 52: 381-89). Standard curve: 1,1,3,3-tetraethoxypropane 0.1-25nmol in 0.3ml.

The **Compounds 1, 3** and **15** reduced iron-induced MDA formation by 50% approximately, at a concentration of 10^{-3}M for each chelator and of 10^{-4}M for ferric chloride.

In another experiment, the **Compounds 3, 7, 9-17** and **26** were examined for their ability to inhibit lipid peroxidation *in vitro* by measuring their capability to inhibit MDA formation in the presence of 10^{-4}M $FeCl_3$ in rat brain homogenates. Ferric chloride(10^{-4}M)-induced lipid peroxidation, as measured by MDA formation in rat cerebral cortex homogenates, was inhibited to a different degree by 10^{-3}M of the various chelators. All compounds tested inhibited MDA formation, but the **Compounds 3, 11-16** and **26** were found to be more effective.

It is important to note that the *in vitro* results may not parallel the *in vivo* anti-oxidant potentials of the chelators but give only an indication of their ability to reduce oxidative stress. Anti-oxidant activity of any drug *in vivo* may be affected by many parameters, e.g. the ability to cross membranes, the interaction with surrounding molecules, the local pH and ionic strength etc.

EXAMPLE 28

Prevention of 6-OHDA-induced toxicity in rats

Out of the iron chelators examined *in vitro* in Example 27, two different types of iron chelators, namely **Compound 3** and **Compound 15**, which were most effective in inhibiting MDA formation, were chosen for *in vivo* studies, in which the chelators (200 µg) were injected intraventricularly in rats alone or prior to 6-OHDA (250 µg).

Male Sprague-Dawley rats, weighing 230-270 g, were housed in a controlled-temperature room with a standardized dark-light schedule (12/12h) for 4 weeks. Rats were anesthetized with a mixture of 15 mg/kg of pentobarbital and 60 mg/kg of chloral hydrate. 6-OHDA (250µg in 5µl of 0.9% NaCl containing 0.2% ascorbic acid), the chelator **3** or **15** (200µg in 5µl), a combination of both (the chelator **3** or **15** 15 min before 6-OHDA), or saline (5µl) (control) was injected into the right cerebral ventricle using stereotactic techniques. The coordinates with bregma as the reference were D 0.8 mm, L 1.3 mm, and V 3.6 mm according to the atlas of Paxinos and Watson. Pargyline (50mg/kg i.p.) and desmethylimipramine-HCl (25mg/kg i.p.) were administered to all the rats 60 min before intracerebroventricular injection. Pargyline inhibits monoamine oxidase and thereby enhances the toxicity of 6-OHDA, and desmethylimipramine provides protection for central noradrenergic neurons from the toxin. All the animals received a daily injection of isotonic glucose (4ml/day i.p.) until they regained their original body weight. Behavioral tests were performed 4 weeks after operation, commencing between 8 and 10 a.m. The rats were killed after the behavioral studies. Desferal was obtained from Ciba Geigy, and other chemicals were from Sigma (St. Louis, MO, U.S.A.).

For behavioral studies, rats were placed on a Varimax activity meter (Columbus Instruments). Horizontal

spontaneous locomotor activity in a novel space was measured during the first 5 min. Rearing activity (spontaneous lifting of the two front paws off the cage floor) was determined every fourth minute for 30 min by direct observation by two individuals blind to the treatment.

Norepinephrine (NE), DA, and metabolite levels were measured as follows: four weeks postoperatively, rats were killed by decapitation, and the brains were rapidly removed. The striata were dissected on an ice-chilled glass plate and quickly frozen in liquid nitrogen. The endogenous levels of NE, DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were determined by HPLC with electrochemical detection (Ben-Shachar et al. (1991) Eur. J. Pharmacol. 202:177-83). All data are expressed as mean±SEM values. Statistical analysis was carried out by analysis of variance with multiple comparisons followed by Student's t test.

Striatal dopamine and its metabolites DOPAC and HVA concentrations, which were determined by HPLC, served as a criteria for the extent of the damage caused by 6-OHDA in the presence or absence of the iron chelators. The specificity of the effects of 6-OHDA and of the chelators **3** and **5** was established by studying the changes in striatal norepinephrine (NE) and serotonine (5-HT) and its main metabolite 5-HIAA (5-hydroxy-indole acetic acid).

Both **Compounds 3** and **15** at a dose of 200µg efficiently prevented the 6-OHDA-induced reduction in striatal dopamine and DOPAC concentrations in the rat. The significant damage caused by 6-OHDA to the nigrostriatal dopamine neurons manifests itself in the increased dopamine turnover which is calculated by the ratio (DOPAC+HVA)/DA. Dopamine turnover was normal in rats pretreated with iron chelators (Table 1).

Table 1: Biogenic amines and their metabolites in the rat striatum after intraventricular injection of 200µg of chelator 3 or 15 prior to 250µg 6-OHDA

pmol/mg tissue	saline (9)	6-OHDA (9)	**15** Comb. (8)	**3** Comb. (8)
NE	4.1±0.2	5.0±0.1	5.01±0.1	4.7±0.5
DA	47.4±2.2	19.93±5.0[c]	33.8±4.3	31.84±5.3
DOPAC	2.31±0.06	1.79±0.25[a]	2.45±0.25	2.15±0.28
HVA	1.96±0.08	2.24±0.23	2.67±0.33	2.68±0.43
5-HT	4.50±0.51	4.00±0.35	4.24±0.43	4.40±0.41
5-HIAA	4.10±0.29	3.76±0.20	4.48±0.38	4.60±0.53
(DOPAC+ HAV)/DA	*0.09*	0.202	0.15	0.15

Number in brackets represents the number of animals in each treatment. Comb. stands for 200µg chelators +250µg 6-OHDA. a - $p<0.05$, b - $p<0.025$, c - $p<0.001$.

Based on confirmation properties of the two iron chelators **3** and **15**, it was considered that **Compound 15** has a better chance to cross the blood-brain-barrier (BBB) and the studies were continued with **Compound 15**. In order to decrease to minimum the possibility of a direct interaction between the chelator and the toxin as a cause for the protection, and to try to find a smaller effective dose of the chelator, 1µg **Compound 15** was injected intraventricularly prior to the injection of 250µg 6-OHDA. Table 2 shows that even at this dose **Compound 15** was effective in preventing 6-OHDA-induced lesion.

Table 2: Biogenic amines and their metabolites in the rat striatum after intraventricular injection of 1µg of chelator 15 prior to 250µg 6-OHDA.

pmol/mg tissue	saline (8)	6-OHDA (7)	**15** Comb. (8)
NE	1.4±0.1	1.1±0.1	1.3±0.12
DA	5.29±6.4	12.93±3.3[a]	62.9±3.13
DOPAC	2.81±0.5	0.76±0.11[a]	2.49±0.13
HVA	2.67±0.18	1.10±0.21[a]	2.77±0.25
5-HT	3.33±0.53	3.22±0.42	4.84±0.45
5-HIAA	5.29±0.53	6.29±0.65	4.98±0.46
(DOPAC±HAV)/DA	0.09	0.14	0.08

Number in brackets represents the number of animals in each treatment. Comb. stand for 1µg chelator **15** +250µg 6-OHDA.
a - $p<0.001$.

The main goal at this stage of research was to find out whether **Compound 15** given peripherally would be able to prevent 6-OHDA-induced toxicity. In other words the question was whether the chelator will stay stable in the periphery, cross the BBB and **Compound 15** (5mg/Kg i.p) for 10 days. Control group received phosphate buffer pH-6.4 0.1M. On the 11th day, the rats of both groups were injected intra-ventricularly with 250µg 6-OHDA. Partial but significant protection against 6-OHDA toxicity was observed with peripheral pretreatment with **Compound 15** (Table 3).

As expected, the neurotoxin 6-OHDA caused an 80% decrease in striatal dopamine levels which was accompanied by a significant decrease in its metabolites DOPAC and HVA. Intraperitoneal treatment with **Compound 15** for 10 days before intraventricular injection of 6-OHDA (combination) partially protected the dopaminergic neurons from degeneration as expressed by dopamine, DOPAC and HVA levels (not shown).

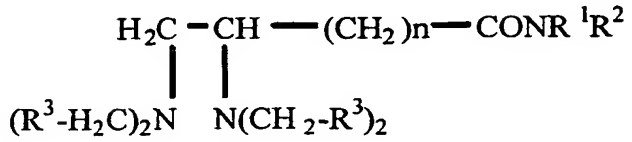
Table 3: Biogenic amines and their metabolites in the rat striatum after chronic peripheral injection of 5 mg/Kg Compound 15 prior to intraventricular injection of 250µg 6-OHDA

pmol/mg tissue	saline (6)	6_OHDA (7)	**15** Comb. (8)
NE	1.09±0.03	1.22±0.04	1.21±0.4
DA	49.2±2.59	9.69±2.63[a]	24.4±4.4[ab]
DOPAC	2.02±0.28	0.51±0.11[A]	1.4±0.25
HVA	2.56±0.22	1.05±0.19[A]	2.28±0.75
5-HT	2.99±0.18	2.60±0.15	2.6±0.31
5-HIAA	1.53±0.09	1.57±0.07	1.59±0.16
(DOPAC+HAV)/DA	0.09	0.16	0.15

Number in brackets represents the number of animals in each treatment. Comb. stand for chelator **15** (5mg/Kg/day i.p. for 10 days) + 250µg 6-OHDA.
a - $p<0.001$ vs. saline; b - $p<0.01$ vs. 6-OHDA.

Appendix A - Structures of compounds I, II and 1-26

I



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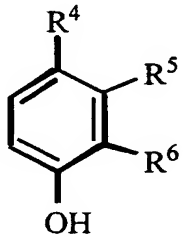

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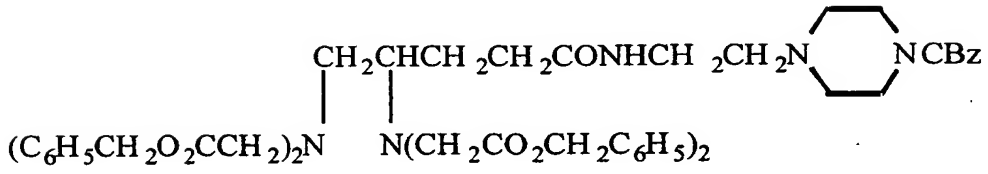

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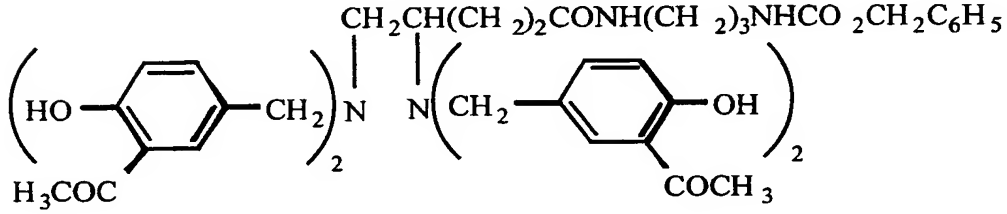

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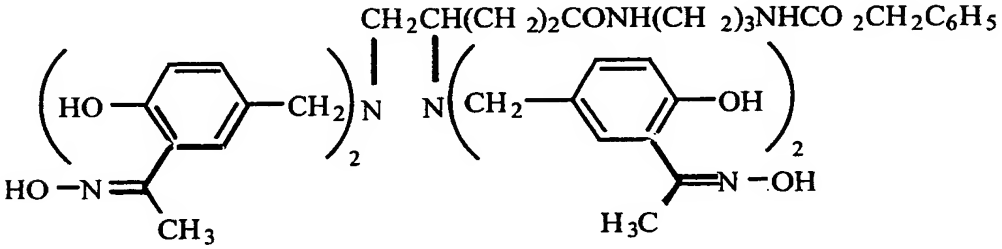

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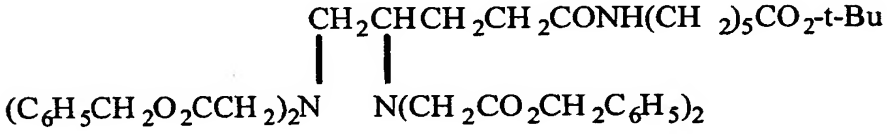

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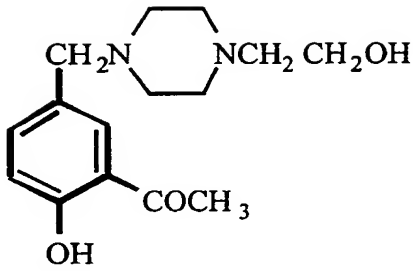

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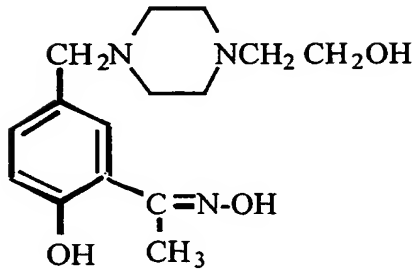

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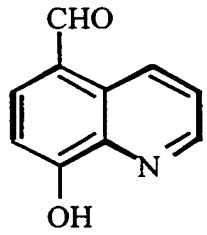

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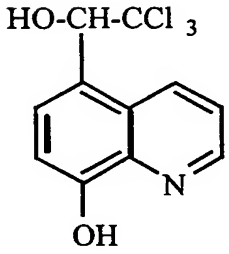

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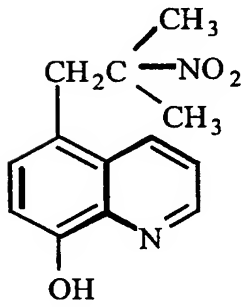

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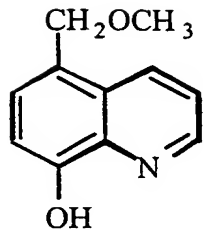

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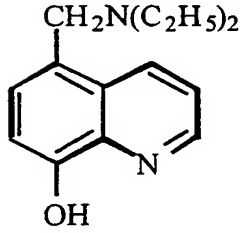

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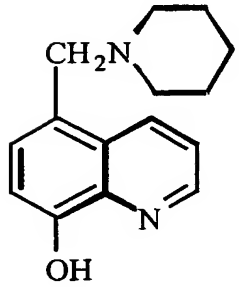

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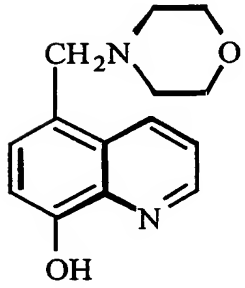

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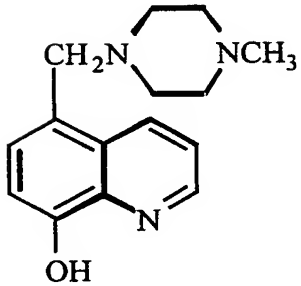

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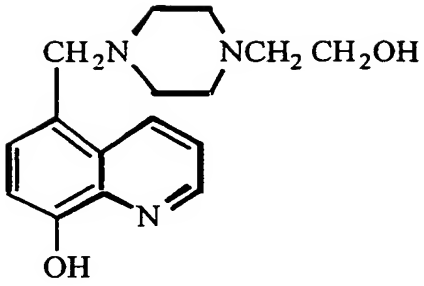

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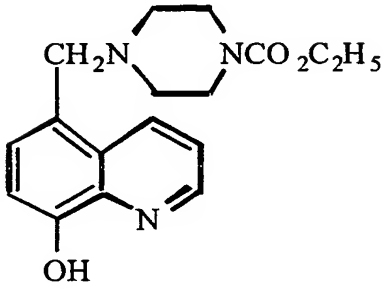

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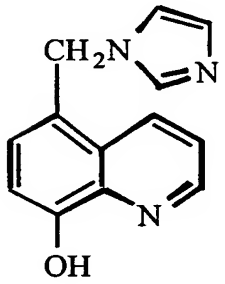

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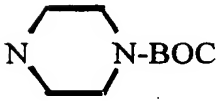

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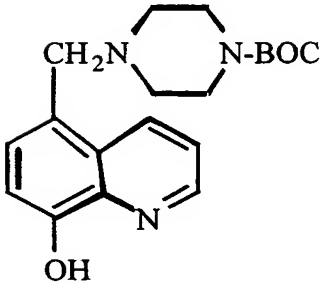

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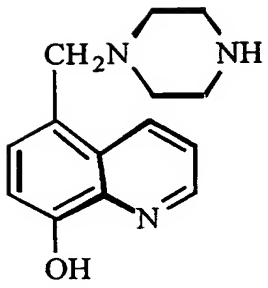

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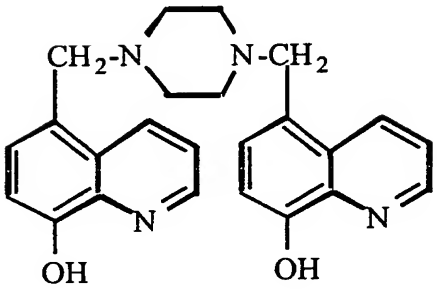

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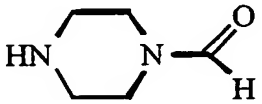

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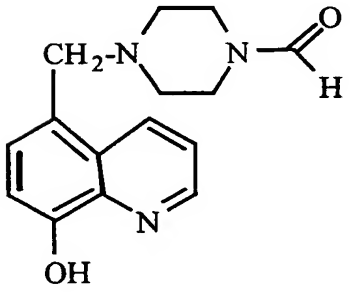

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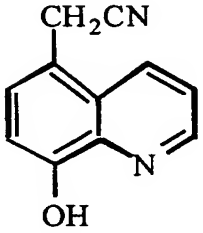

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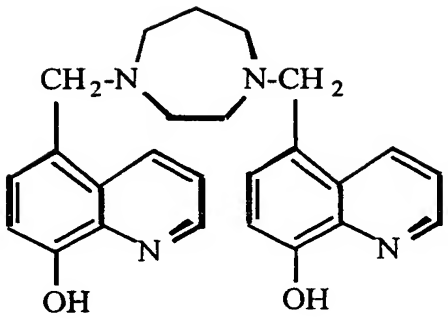

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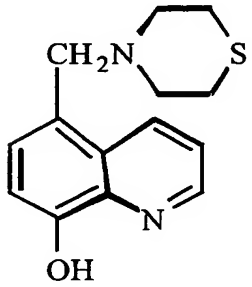
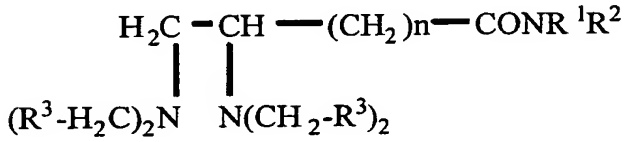


CLAIMS

1. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound selected from the group consisting of:

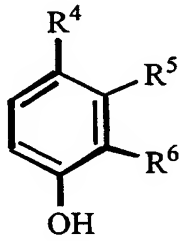
(a) a compound of formula I:



wherein

R^1 is H or hydrocarbyl; R^2 is a hydrophobic radical; R^3 is a radical selected from 3-(C_2-C_6)acyl-4-hydroxyphenyl, 3-hydroxyimino(C_2-C_6)alkyl-4-hydroxyphenyl, or COOZ, wherein Z is H, (C_1-C_6)alkyl, aryl or ar(C_1-C_6)alkyl; and n is an integer from 1 to 20; and

(b) a compound of formula II:



wherein

R^4 is (C_1-C_6)acyl, nitro(C_1-C_6)alkyl, cyano(C_1-C_6)alkyl, (C_1-C_6)alkoxy(C_1-C_6)alkyl or $-CH_2NR^7R^8$, wherein R^7 and R^8, the same or different, is each H or (C_1-C_6)alkyl, or together with the N atom form a saturated or unsaturated 5-7 membered ring optionally containing a further heteroatom selected

from N, O or S, the further N atom in such saturated 5-7 membered ring being optionally substituted by C_1-C_6 alkyl, C_1-C_6 acyl, hydroxy-(C_1-C_6)alkyl, (C_1-C_6)alkoxycarbonyl, and 8-hydroxyquinolin-5-yl-(C_1-C_6)alkyl,

and

either R^5 is H and R^6 is (C_2-C_6) acyl or hydroxyimino(C_2-C_6)alkyl, or R^5 and R^6 together with the phenyl ring form a quinoline, a 1,2,3,4-tetrahydroquinoline or a perhydroquinoline ring,

and

pharmaceutically acceptable salts of the compounds of formulas I and II.

2. A pharmaceutical composition according to claim 1, comprising a compound of formula I wherein n is 2 to 4, preferably 2; R^1 is H or a saturated, unsaturated or aromatic hydrocarbyl radical, preferably selected from C_1-C_8 alkyl, C_2-C_8 alkenyl and phenyl; R^2 is a hydrophobic radical selected from C_6-C_{20} alkyl, C_6-C_{20} alkenyl, a radical selected from C_5-C_{20} acyl, benzyloxycarbonyl, substituted benzyloxycarbonyl, C_3-C_8 alkoxycarbonyl, cycloalkoxy-carbonyl and aryloxycarbonyl, said radical being either linked directly to the N atom or through a (C_1-C_5) alkylene chain, and N-substituted amino or 4-substituted-piperazino linked to the N atom through a (C_1-C_5) alkylene chain; and R^3 is a radical selected from 3-(C_2-C_6)acyl-4-hydroxyphenyl, 3-hydroxyimino(C_2-C_6)alkyl-4-hydroxyphenyl, or COOZ, wherein Z is H, (C_1-C_6)alkyl, aryl or ar(C_1-C_6)alkyl.

3. A pharmaceutical composition according to claim 2, wherein R^2 is straight or branched C_6-C_{20} alkyl or alkenyl; saturated or unsaturated C_5-C_{20} carboxylic acyl linked directly to the N atom or through a (C_1-C_5) alkylene chain; benzyloxycarbonyl or halo-substituted benzyloxycarbonyl,

such as o- and p-chloro-benzyloxycarbonyl, 2,4- and 2,6-dichlorobenzyloxycarbonyl, linked directly to the N atom or through a (C_1-C_5) alkylene chain; a bulky alkoxycarbonyl group such as tert-butoxycarbonyl linked directly to the N atom or through a (C_1-C_5) alkylene chain; cycloalkoxycarbonyl linked directly to the N atom or through a (C_1-C_5) alkylene chain; aryloxycarbonyl such as fluorenylmethoxycarbonyl, linked directly to the N atom or through a (C_1-C_5) alkylene chain; 4-substituted-piperazinyl or N-substituted amino, linked to the N atom through a (C_1-C_5) alkylene chain, wherein the 4- and N-substituent is a hydrophobic group selected from C_6-C_{20} alkyl, C_6-C_{20} alkenyl, C_5-C_{20} acyl, benzyloxycarbonyl, substituted benzyloxycarbonyl, C_3-C_8 alkoxycarbonyl, cycloalkoxycarbonyl, aryloxycarbonyl, N-substituted amino and 4-substituted-piperazinyl, all such substituents being as defined above.

4. A pharmaceutical composition according to claim 3, wherein n is 2, R^1 is H, R^2 is a radical $-(CH_2)_3NHCOOCH_2C_6H_5$, 5-(tert-butoxycarbonyl)pentyl, or $-(CH_2)_2$-(4-carbobenzoxy)-piperazinyl, and R^3 is benzyloxycarbonyl, 3-(1-hydroxy-iminoethyl)-4-hydroxyphenyl or 3-acetyl-4-hydroxyphenyl.

5. A pharmaceutical composition according to claim 4, comprising a compound of formula I selected from:

N-[2-(4-carbobenzoxypiperazin-1-yl)ethyl]-4,5- bis[bis (benzyloxycarbonylmethyl)amino]valeramide (1)

N-(3-benzyloxycarbonylaminopropyl)-4,5-bis[bis (3-acetyl-4-hydroxybenzyl)amino]valeramide (2)

N-(3-benzyloxycarbonylaminopropyl)-4,5-bis[bis(3-(1-hydroxy-iminoethyl)-4-hydroxybenzyl)amino]valeramide (3)

N-[5-(tert-butyloxycarbonyl)pentyl]-4,5-bis[(bis (benzyloxycarbonyl)methyl]amino]valeramide (4)

6. A pharmaceutical composition according to claim 1, comprising a compound of formula II wherein R^4 is C_1-C_6 acyl, nitro(C_1-C_6)alkyl in which the (C_1-C_6)alkyl group may be branched, cyano(C_1-C_6)alkyl, preferably cyanomethyl, (C_1-C_6) alkoxy(C_1-C_6)alkyl, preferably methoxymethyl, or $CH_2NR^7R^8$, in which R^7 and R^8 are both H, or one is H and the other is (C_1-C_6) alkyl, or both R^7 and R^8 are C_1-C_6 alkyl, or R^7 and R^8 together with the N-atom form a saturated or unsaturated 5-7 membered ring optionally containing a further heteroatom selected from N, O or S, the further N-atom in such saturated 5-7 membered ring being optionally substituted by (C_1-C_6) alkyl, (C_1-C_6) acyl, hydroxy-(C_1-C_6)alkyl, (C_1-C_6) alkoxycarbonyl, and 8-hydroxyquinolin-5-yl(C_1-C_6) alkyl, preferably 8-hydroxyquinolin-5-yl-methyl.

7. A pharmaceutical composition according to claim 6, wherein R^4 is a radical selected from formyl, 2-methyl-2-nitropropyl, cyanomethyl, methoxymethyl, (diethyl)amino-methyl, piperidinomethyl, morpholinomethyl, thiomorpholino-methyl, piperazinomethyl, imidazolylmethyl, 4-methyl-piperazinomethyl, 4-(2-hydroxyethyl)piperazinomethyl, 4-formylpiperazinomethyl,4-(ethoxycarbonyl)piperazinomethyl, 4-(butoxycarbonyl)piperazinomethyl, 4-(8-hydroxyquinolin-5-yl-methyl)-piperazinomethyl, and 4-(8-hydroxy-quinolin-5-yl-methyl)homopiperazinomethyl.

8. A pharmaceutical composition according to claim 6 or 7, comprising a compound of formula II wherein R^5 is H and R^6 is (C_2-C_6) acyl, preferably acetyl, or hydroxyimino(C_2-C_6)alkyl, preferably hydroxyiminoethyl.

9. A pharmaceutical composition according to claim 8, comprising a compound of formula II selected from:

2-acetyl-4-[4-(2-hydroxyethyl)piperazin-1-yl-methyl] phenol (5)

2-(1-hydroxyiminoethyl)-4-[4-(2-hydroxyethyl)piperazin -1-ylmethyl]phenol (6)

10. A pharmaceutical composition according to claim 6 or 7, comprising a compound of formula II wherein R^5 and R^6 together with the phenyl ring form a quinoline ring.

11. A pharmaceutical composition according to claim 10, comprising a quinoline compound selected from:

5-formyl-8-hydroxyquinoline (7)

5-(2-methyl-2-nitropropyl)-8-hydroxyquinoline (9)

5-methoxymethyl-8-hydroxyquinoline (10)

5-diethylaminomethyl-8-hydroxyquinoline (11)

5-piperidinomethyl-8-hydroxyquinoline (12)

5-morpholinomethyl-8-hydroxyquinoline (13)

5-(4-methylpiperazinomethyl)-8-hydroxyquinoline (14)

5-[4-(2-hydroxyethyl)piperazinomethyl]-8-hydroxy-quinoline (15)

5-[4-ethoxycarbonylpiperazinomethyl)-8-hydroxy-quinoline (16)

5-(imidazol-1-ylmethyl)-8-hydroxyquinolin (17)

5-(4-Boc-piperazinomethyl)-8-hydroxyquinoline (19)

5-piperazinomethyl-8-hydroxyquinoline (20)

N.N'-di-(8-hydroxyquinolin-5-ylmethyl) piperazine (21)

5-(4-formylpiperazinomethyl)-8-hydroxyquinoline (23)

5-cyanomethyl-8-hydroxyquinoline (24)

N.N'-di-(8-hydroxyquinolin-5-ylmethyl)homopiperazine, and

5-thiomorpholinylmethyl-8-hydroxyquinoline (26)

12. A pharmaceutical composition according to any one of claims 1 to 11 for prevention of lipid peroxidation in the brain of mammals.

13. A pharmaceutical composition according to any one of claims 1 to 12 for the treatment of stroke.

14. A pharmaceutical composition according to any one of claims 1 to 12 for the treatment of Parkinson's disease.

15. Use of a compound of formula I or formula II according to any one of claims 1 to 11 for the preparation of a pharmaceutical composition for prevention of lipid peroxidation in the brain of mammals.

16. Use of a compound of formula I or formula II according to any one of claims 1 to 11 for the preparation of a pharmaceutical composition for the treatment of stroke.

17. Use of a compound of formula I or formula II according to any one of claims 1 to 11 for the preparation of a pharmaceutical composition for the treatment of Parkinson's disease.

18. A compound of formula I in claim 1, excepting the compound N-[5-(tert-butoxycarbonyl)pentyl]-4,5-bis[(bis (benzyloxycarbonyl)methyl]amino]valeramide.

19. A compound of formula II in claim 1, excepting the compounds 5-formyl-8-hydroxyquinoline and 5-methoxymethyl-8-hydroxyquinoline.

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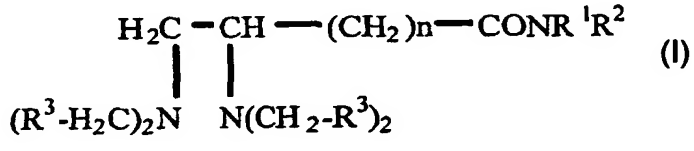
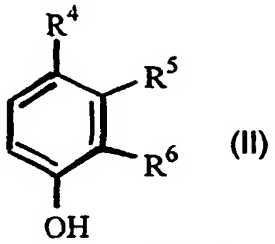
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NOVEL IRON CHELATORS AND PHARMACEUTICAL COMPOSITIONS COMPRISING IRON CHELATORS FOR THE TREATMENT OF NEURODEGENERATIVE DISORDERS



(I)



(II)

(57) Abstract: Use of a compound of formula (I), wherein R^1 is H or hydrocarbyl; R^2 is a hydrophobic radical; R^3 is 3-(C_2-C_6)acyl-4-hydroxyphenyl, 3-hydroxyimino(C_2-C_6)-alkyl-4-hydroxyphenyl, or COOZ, wherein Z is H, (C_1-C_6)alkyl, aryl or ar(C_1-C_6)alkyl; and n is 1-20; and of a compound of formula (II), wherein R^4 is (C_1-C_6)acyl, nitro(C_1-C_6)alkyl, cyano(C_1-C_6)alkyl, (C_1-C_6)alkoxy(C_1-C_6)alkyl or $-CH_2NR^7R^8$, wherein R^7 and R^8, the same or different, is each H or (C_1-C_6)alkyl, or together with the N atom form a saturated or unsaturated 5-7 membered ring optionally containing a further heteroatom selected from N, O or S, the further N atom being optionally substituted, and either R^5 is H and R^6 is (C_2-C_6) acyl or hydroxyimino(C_2-C_6)alkyl, or R^5 and R^6 together with the phenyl ring form a quinoline, a 1,2,3,4-tetrahydroquinoline or a perhydroquinoline ring, for the preparation of pharmaceutical compositions for the treatment of Parkinson's disease or stroke.

WO 00/74664 A3

INTERNATIONAL SEARCH REPORT

Interr. nal Application No
PCT/IL 00/00332

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/165 A61K31/137 A61K31/15 A61K31/47 A61K31/4709
A61P25/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBASE, MEDLINE, BIOSIS, CHEM ABS Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KAHANA N ET AL: "A CONCEPTUAL APPROACH TO THE SYNTHESIS OF BIFUNCTIONAL EDTA ANALOGSEDTA-EXTENDED POLYAMIDES" JOURNAL OF ORGANIC CHEMISTRY,US,AMERICAN CHEMICAL SOCIETY. EASTON, vol. 59, no. 17, 26 August 1994 (1994-08-26), pages 4832-4837, XP000576114 ISSN: 0022-3263 * See scheme 3, compounds N. 5 * ---	1-5, 12-14
Y	WARSHAWSKY A.: "Bifunctional Chelating Agents Part 3." J. CHEM. SOC. PERKIN TRANS. I, vol. 10, 1989, page 1781-6 XP002155815 * See compounds in figure at page 1783 * ---	1-5, 12-14

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Veronese, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 329 481 A (NEORX CORP) 23 August 1989 (1989-08-23) claims; figures 2A,2B,3A,3B, ---	1-5, 12-14
A	HALL E D ET AL: "Neuroprotective efficacy of microvascularly-localized versus brain-penetrating antioxidants." ACTA NEUROCHIRURGICA. SUPPLEMENTUM, (1996) 66 107-13. REF: 23, XP000972206 figure 1; table 1 ---	1-5, 12-17
A	US 4 652 519 A (WARSHAWSKY ABRAHAM ET AL) 24 March 1987 (1987-03-24) the whole document ---	1-5, 12-14
A	WESEMANN, W. (1) ET AL: "Effect of lazaroid U-74389G on iron -induced reduction of striatal dopamine metabolism." JOURNAL OF NEURAL TRANSMISSION SUPPLEMENT, (1995) VOL. 46, NO. 0, PP. 175-182., XP000972216 cited in the application the whole document ---	1-5, 12-17
A	BEN-SHACHAR D ET AL: "IRON MELANIN INTERACTION AND LIPID PEROXIDATION IMPLICATIONS FOR PARKINSON'S DISEASE." J NEUROCHEM, (1991) 57 (5), 1609-1614., XP000972207 the whole document ---	1-5, 12-17
X	WARSHAWSKY A ET AL: "Cytotoxicity effects of transition-metal chelators of the 5-substituted 2-hydroxyacetophenones and their oximes." EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, vol. 30, no. 7-8, 1995, pages 553-560, XP002163945 ISSN: 0223-5234 tables 1,2 --- -/--	1,6-8, 12-14,19

INTERNATIONAL SEARCH REPORT

Intern ial Application No
PCT/IL 00/00332

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KIRIENKO, G. K. ET AL: "Derivatives of 8-hydroxyquinoline as possible anthelmintics, nematocides, and fungicides" retrieved from STN Database accession no. 70:106350 HCA XP002163950 abstract & IZV. AKAD. NAUK MOLD. SSR, SER. BIOL. KHIM. NAUKI (1967), NO. 10, 55-62 FROM: REF. ZH., KHIM. 1968, ABSTR. NO. 15N536.,	1,6,7, 10-14,19
X	JP 63 238060 A (MARUHO KK) 4 October 1988 (1988-10-04) abstract	1,6,7, 10-14,19
X	WARNER V D ET AL: "Quantitative structure -activity relationships for 5-substituted 8- hydroxyquinolines as inhibitors of dental plaque." JOURNAL OF MEDICINAL CHEMISTRY, (1977 JAN) 20 (1) 92-6. , XP002163946 table 1	1,6,7, 10-14
X	WARNER V D ET AL: "Synthesis and in vitro evaluation of 8- hydroxyquinoline analogs as inhibitors of dental plaque." JOURNAL OF MEDICINAL CHEMISTRY, (1976 JAN) 19 (1) 167-9. , XP002163947 figures; tables	1,6,7, 10-14,19
X	BURCKHALTER, JOSEPH H. ET AL: "Amino - and chloromethylation of 8- quinolinol -mechanism of preponderant ortho substitution in phenols under Mannich conditions" JOURNAL OF ORGANIC CHEMISTRY, vol. 26, October 1961 (1961-10), pages 4078-4083, XP002163948 page 4081	1,6,7, 10-14,19
X	MATSUMURA, KONOMU ET AL: "Condensation of chloral hydrate with 8- quinolinol" JOURNAL OF THE AMERICAL CHEMICAL SOCIETY, 1955, pages 6671-6674, XP002163949 the whole document	1,6,7, 10-14

8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 00/00332

B x I Observations where certain claims w r found unsearchable (Continuati n of it m 1 f first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

B x II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompani d the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/IL 00 00332

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: Claims 1,12-17, (partial); 2 - 5, 18 (complete).

   Pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound having the formula I shown in claim 1.

2. Claims: Claims 1, 12-17, (partial); 6-11, 19 (complete).

   Pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound having the formula II shown in claim 1.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/IL 00 /00332

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1,6,7,8,12-18 relate to an extremely large number of possible compounds/compositions. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds/compositions claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds explicitally disclosed at page 37-41 of the application, with due regard to the general idea underlying the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern ial Application No
PCT/IL 00/00332

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0329481 A	23-08-1989	US 5202451 A	13-04-1993
		JP 2152956 A	12-06-1990
		US 5606028 A	25-02-1997
US 4652519 A	24-03-1987	NONE	
JP 63238060 A	04-10-1988	NONE	